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THE EVOLUTION OF STABILITY IN MARINE ENVIRONMENTS NATURAL SELECTION AT THE LEVEL OF THE ECOSYSTEM

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One of the most striking contrasts between the lower and the higher latitudes is manifested by the stability of the warm-adapted floras and faunas and the instability of the ecosystems of the cooler parts of the world. The argument developed here is that this contrast suggests, among other things, that selection may apply at the level of the ecosystem as well as at the levels of the individual and the specific population. Ecosystems can compete, and evolution of the stable ecosystem can be looked upon as a process of learning, analogous to the learning of regulated behavior in the nervous systems of animals.

OSCILLATIONS IN NUMBER

In this discussion, I am starting from the premise that oscillations are bad for any system and that violent oscillations are often lethal. The more violent the oscillation in specific numbers in any ecological situation, the greater the danger of extinction of species, at least of local extinction, causing serious disturbance of the community, possibly the extinction of the whole system, again locally.

Population oscillations of fairly wide amplitude are well known in terrestrial environments among rodents and their predators, game birds, rabbits, and insects, and much study has been devoted to them. Oscillations of this sort, in which a primary oscillator, a herbivore, causes sympathetic oscillations in carnivore populations which in turn take part (however small a part) in reducing the amplitude of the oscillations of the herbivore, appear only in relatively simple ecosystems, containing a limited, usually quite small, number of species. Such simplicity is found as a rule only in cool climates with marked seasonal variation, that is to say, in temperate and polar climates. (A special case might be made here for desert regions, in which ecosystems are also simple.) Oscillations of this sort are absent in tropical and subtropical environments, which foster much more complex ecosystems in which there is great multiplicity of energy paths along which overloadings can be released, with consequent great decrease or virtual elimi-

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nation of the time-lag effect which is largely responsible for the oscillations in the simpler systems (Hutchinson, 1948; Riley, 1953; Odum, 1959; MacArthur, 1955).

The simplicity of the ecosystems in cool and cold climates is presumably partly or mainly a result of the novelty of the present polar conditions. Whether or not we accept the views of Zenkevitch (1949) on the ancient origin of the cold-adapted fauna or the more conservative estimates of the age of the present polar cooling of Barghoorn (1953) and Durham (1950), there can be little doubt that the conditions existing during the Pleistocene were special. The short million years since the end of the Pliocene, or the few million years of glacial climate possibly since some time in the Pliocene or Miocene, and the much shorter time available for colonization of areas actually glaciated, have not been long enough to allow the adaptation to the new conditions towards the poles of more than a relatively small number of species. Such adapted forms have by establishing themselves first, further lowered the chances of successful establishment by others, the more so because the number of possible niches and habitats in the colder regions is very much less than in warmer parts of the world.

SEASONAL OSCILLATIONS

What is true of fluctuations whose periods occupy a few years is also true of *seasonal* oscillations—they are most marked in temperate and polar regions, and absent or poorly developed in the tropics both on land and in the sea. In the plankton, for instance, upon whose numerical behavior everything else in the sea depends, production is continuous at a steady controlled level all year round in tropical waters, according to Steemann Nielsen (1957). Plant growth on land, in equatorial regions, has a similar year-round activity; individual species may develop periodicities of flowering and of new leaf growth, not necessarily annual periodicities, but the total mass and the total activity level remain fairly constant (Holttum, 1953). Steemann Nielsen describes extraordinary stability in the planktonic system in tropical seas. The phytoplankton does not at any time completely consume the available phosphate and nitrate supplies, which are continuously present at low levels; the zooplankton does not deplete the phytoplankton, and predation upon the zooplankton does not, apparently, cause oscillations. This means that the levels of production are delicately controlled in a hydrographic system which is itself highly stable. Except for certain areas of large-scale upwelling, which are among the most productive regions in the world, the warmer seas are very stable, and vertical movements of water are effectively and stubbornly resisted. The supply of nutrient salts is thus limited to (1) regeneration within the euphotic layer, and (2) such quantities as can enter any given area from outside by horizontal transport. These sources have to maintain the equilibrium against constant loss from the system of salts contained in detritus which sinks out of the photosynthetic layer before it is mineralized. The quantities involved in this loss are not yet known.

The contrast between this tropical stability and the sharply oscillating annual cycles of standing crop in temperate and polar systems is obvious, and it is equally clear that the greatly differing climatic conditions are intimately associated with the contrast. The annual variation of light intensity and angle of incidence, the larger amplitude of annual temperature variation, and the unstable condition of the colder waters in winter, are all involved here. It may not be so obvious, however, that these annual oscillations in both marine and terrestrial ecosystems have something important in common with the "fur-bearer" type of oscillations already mentioned. Both types can be attributed to climate; the former as just described and the latter owing to the simplicity of the ecosystem, which is itself a result of climatic demands. Both groups of oscillations are to a high degree the result of the onset of the glacial climate. On the original premise, then, that oscillations are disadvantageous both to the individual species and to the ecosystem as a whole, I conclude that the steady systems of the tropics are the result of long evolution and that oscillations observed in the higher latitudes are systems of non-adaptation. Arctic and antarctic faunas are immature, still in the elementary stages of evolutionary "learning."

ADAPTATIONS TO COLD CLIMATES

The next thing to look for are signs of incipient adaptation, in this ecological sense, to life in cold climates. Adaptation in this case would be a matter of changes in the system which would tend to (1) increase the complexity of the ecosystem, that is, increase the number of species involved; (2) lower the rates of production from the maxima possible in both plants and animals; (3) spread the rate of grazing upon the plants as evenly as possible over the 12 months of the year, and (4) carry these processes up to higher trophic levels. This would involve control of the breeding rates of both plant and animal populations, and possibly also the regulation of the manner in which individuals in the herbivore and carnivore populations grow, and of their energy requirements. There is evidence that processes of this sort have in fact been evolved, or are in process of evolution.

1. The findings of Thorson (1950, 1952) can profitably be interpreted in this light. Thorson found that in marine shallow water benthos in temperate and arctic regions, the pelagic larva may or may not be retained. If it is retained, the spawning is restricted to a short period in the spring calculated to coincide with the abundance of the spring plankton; if it is not, the spawning period is much longer and could extend throughout the year. There is a direct relationship between the mean temperature of the environment and the proportion of species which retain the pelagic larva. The stabilizing effect of the loss of larva and production of larger eggs is thus best developed in the colder regions.

2. Steele (1959), working on the eastern Canadian arctic shallow water Amphipoda, concludes that whereas the littoral species have developed short seasonal breeding periods in the spring or summer, the benthonic forms as a rule breed all the year round. The immediate or proximate reason

for this may well be that the detrital or bacterial food of these species is present the year round anyway; nevertheless, the ecological effect is towards high biomass in winter.

3. Recent work on the marine fauna of the arctic regions has brought to light the fact that many species produce their young not simply at any time of year, but specifically in the dead of winter. In a situation in which the spring bloom of phytoplankton is sudden and violent, in which there may be no fall bloom at all and the winter stock of plant food tends to be quite small, so far as we know, one would have expected that all the animal species which depended on phytoplankton in the younger stages would have evolved a breeding cycle that would release the young at the most propitious moment, at some time between the beginning and the peak of the spring phytoplankton bloom. Many species of course do this, notably the copepods, but there are many that do not, and as the study of arctic breeding cycles advances, more such species appear. MacGinitie (1955) gives many examples of invertebrate species in the waters of the Point Barrow area, Alaska, which begin to develop their eggs in October and later, and many which produce ripe eggs at that time; some of the amphipods had hatched young in the marsupium. Dunbar (1957) records the dominant arctic pelagic amphipod *Themisto libellula* as maturing late in the autumn or in the early winter and carrying hatched young in December. *Gammarus setosus* breeds in the colder part of the year, and differs in this respect from its close relative *G. oceanicus* (MacIntyre, personal communication). And among the 114 species of amphipods taken in Ungava (Dunbar, 1954), making up a very large material collected over four seasons, only 55 species were found to include ovigerous or paedigerous females during the spring and summer months, from June to September. The material collected by the Russian drifting station expedition in the polar basin in 1950–1951 included several species recorded as spawning in winter (Brodskii and Nikitin, 1955). This winter breeding has the effect of increasing the number of species in the system. This is analogous to the adoption of nocturnal or diurnal habit, and it also spreads the rate of predation on the available food more evenly over the year.

4. There is something possibly significant in this regard in the large size and lowered growth rate of arctic poikilotherms. Harvey (1955), in discussing growth and metabolism in marine poikilotherms, writes: "In addition to this general inverse relation between age or size of animals and losses by respiration, there is an inverse relation between age and growth rate (the daily percentage increase in organic matter). It appears usual that growth rate decreases more rapidly with increasing size than respiration decreases.... In consequence, a greater proportion of food assimilated by young animals is built into new tissue than by old animals. Hence the same rate of plant production may permit a greater biomass of a stable community consisting mostly of aged, larger, slow-growing, slow-respiring animals than of one mainly composed of small quick-growing animals. The latter fauna, however, may synthesise more animal tissue yearly, the rate of turnover of living tissue in the animals being greater."

This, the development of large size and slow growth and metabolic rates, is precisely what happens in cold polar waters, which raises the question whether this situation may not be the result of adaptation to the total ecological condition, assuming that there is selection favoring stability.

5. The two-phase or alternating breeding cycle demonstrated in certain cold-water members of the zooplankton (for example, Dunbar, 1957), such as *Themisto libellula*, *Sagitta elegans*, *Thysanoessa* spp., may also be interpreted in this light, since it leads to stability of the species population and maintenance of large numbers of individuals under conditions of slow growth, slow maturation and probably limited food supply. In this type of cycle two broods coexist in the same body of water, the two broods being separated in time so that they may be partially or completely isolated from each other reproductively.

6. Finally, there is an example of stability which is as striking as that of the tropical plankton, and about which a little more is known. The families of oceanic birds are remarkable for the stability of their population numbers. The range of a species may increase or decrease over a period of years, as in the case of the Fulmar Petrel in the British Isles or of the Gannet in the North Atlantic, but oscillations of the sort discussed here do not appear to occur. They breed on small island groups or rock cliff shores, with a food supply that must be considered to be virtually without limit for practical purposes, and with great mobility. In spite of this plenty, the oceanic birds are most modest in their breeding rates. The numbers of eggs laid are small, often only one per pair, and breeding may not occur every year per individual, at least in some species. Why this frugality amid such plenty? I suggest that these are highly evolved stable populations which have in the past been subjected to the stress of oscillation in an oscillating system, and that they have responded to selection for this self-regulating character of a restricted breeding rate, tending toward stability. Baker (1938) quotes examples of many tropical (terrestrial) birds which lay only two eggs in a clutch, though they belong to genera in which five or six eggs are the rule among temperate representatives.

Some of the sea-bird populations mentioned above breed in polar or temperate regions. But if they do, they are migrants, and are present toward the poles only when the food supply is maximal for the year. As such they serve to cut down the animal marine populations when the latter are high, and depart for other parts when the food supply grows less; they do not prey upon animals during the low-level period in the oscillation. Moreover, birds, as homotherms, are less affected physiologically by the polar climates than are the poikilotherms—the problems of adaptation are much simpler. This is true also of the sea mammals, whose numbers also appear to be steady. Certain of the most numerous sea mammals that breed in the north are migratory; the phenomenon of migration itself may perhaps profitably be considered as the result of evolution at the ecological or ecosystem-level, as something which tends to stabilize both the systems of the breeding areas and those of the wintering areas.

The case made here, then, is that the stable non-oscillating system is the "ideal" and the product of a long process of evolution; and that the oscillating systems of regions affected by the present glacial climate of the world are in process of evolution towards better adjustment.

SELECTION OF ECOSYSTEMS

As to the mechanisms by which selection might take effect at this level, they are of the ordinary Darwinian sort except that the criterion for selection is survival of the system rather than of the individual or even the species. For instance, suppose an ecosystem, locally defined, begins to develop oscillations to a lethal degree, a degree such that one or more vital parts are not able to survive; the resulting empty environmental space, as in Cuvierian cataclysms, is available for occupation by communities from the adjacent regions; and these adjacent systems, as their survival suggests, are not of precisely the same constitution as the extinguished system. Perhaps a drift effect has occurred in certain species, or some other isolation effect. One or more of the specific elements will have growth rates, breeding potential and/or metabolic adjustment to temperature different from the former system; and if the difference is favorable to the continued survival of the system, its chances of survival are enhanced. In this way the system dominant in any geographic region changes, and changes (if the present assumptions are correct) in the direction of greater stability.

In this sort of selection, characters which may be of immediate advantage to the species are not necessarily selected into the stock in the long run, as may happen to the character which may be advantageous to the individual but ultimately lethal to the species. A breeding rate high enough to give one species an immediate advantage over another may ultimately prove to be lethal to the whole system. It thus becomes possible, in fact necessary, for selection to favor *slower* growth rate, *longer* time to reach maturity, *winter* spawning, and so on, under certain determining circumstances. It should be pointed out here that the model suggested in this paper has already been called for, as it were, by Hutchinson (1957) who writes (pp. 424-425): "So far little attention has been paid to the problem of changes in the properties of populations of the greatest demographic interest.... A more systematic study of evolutionary change in fecundity, mean life span, age and duration of reproductive activity and length of post-reproductive life is clearly needed. The most interesting models that might be devised would be those in which selection operated in favor of low fecundity, long pre-reproductive life and on any aspect of post-reproductive life." There is much more in the paper just quoted of direct relevance to the thesis put forward here. Slobodkin (1953), also, has pointed out that in certain circumstances a low rate of reproduction may have a greater selective advantage than a high rate. Utida (1957) comes to the conclusion from the mathematical approach that selection in favor of dampening of oscillations is to be expected, and points out that both Hutchinson (1954) and Slobodkin (1953) had come to similar conclusions from other directions. Chitty (1957) describes a non-

infectious pathological condition which develops in *Microtus agrestis* under stress of crowding, and adds: "The main theoretical difficulty is to explain why such a deleterious condition, if controlled by an hereditary factor, has not been eliminated by natural selection." Finally, and in the contrary sense, Barnes (1958), describing the synchronization of spawning in *Balanus balanoides* with the spring phytoplankton outburst, regards such synchronization in arctic regions as essential; it is in fact essential only within the framework of classical selection theory, and it is clear that there are many forms in which no apparent effort at such synchronization is made.

There are probably strict limits to the degree to which an ecosystem can stabilize itself in an environment as variable (seasonally) as the climates of the higher latitudes. Perhaps stability comparable to the tropical stability was not achieved in higher latitudes even before the onset of the glacial climates. The thesis here is simply that there is a selection acting constantly in favor of greater stability, whether or not such selection meets a situation of equilibrium with an oscillating environment, beyond which greater stability of the system may become impossible.

SUMMARY

Starting from the premise that oscillations are dangerous for any system and that violent oscillations may be lethal, this paper contrasts the highly stable production systems of tropical waters with the seasonal and longer-term oscillations of temperate and polar waters. The differences are climatically determined, and since the present glacial type of climate is young in the climatic history of the earth, the ecological systems of the higher latitudes are considered as immature and at a low level of adaptation. That they may be in process of evolution toward greater stability is suggested by a number of phenomena, such as the development of large, slow-respiring, slow-growing individuals, and the production of the young in many arctic invertebrates in mid-winter or late fall. These and other observed peculiarities of high latitude fauna tend to make the most efficient use of the available plant food and to spread the cropping pressure over as much of the year as possible. Oceanic birds are cited as examples in which stable populations have been achieved by evolution of lower breeding rates, and the phosphate and nitrate cycles in the upper layers of tropical seas are discussed. It is emphasized that selection here is operating at the level of the ecosystem; competition is between systems rather than between individuals or specific populations.

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THE RANGE AND PATTERN OF INSECT ABUNDANCE

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In the past twenty years there has been an increasing interest in the relative abundance of different animals—including insects—in mixed wild population.

The actual abundance of any one species at any moment, or in other words the total numbers of individuals, is a synthesis of all the effects, in the preceding months or years, of both physical and biological environments—temperature, rainfall, food supply, enemies, etc. The pattern of the relative abundance of a number of species interlocking in a particular community is therefore a synthesis of all the syntheses of all the species. It is in fact so complex that it may perhaps become amenable to statistical methods of study.

Early quantitative work showed that, in any random sample of individuals taken from a mixed wild community under natural field conditions, there were on the one hand a large number of species with very few individuals, while the total number of individuals was dominated by a few abundant species. More accurate work showed that, in general, the larger the number of individuals per species, the fewer the number of species at that level.

TABLE 1

Macro-Lepidoptera captured in a light trap at Harpenden, England, in the year 1935

Individuals per species	Species	Individuals per species	Species	Individuals per species	Species	Individuals per species	Species
1	37	11	2	21	4	31	...
2	22	12	4	22	1	32	...
3	12	13	2	23	1	33	2
4	12	14	3	24	...	34	2
5	11	15	2	25	1	35	...
6	11	16	2	26	...	36	...
7	6	17	4	27	...	37	...
8	4	18	2	28	2	38	1
9	3	19	...	29	2	39	1
10	5	20	4	30	...	40	3

And also at 42 (2); 48 (2); 51; 52; 53; 58; 61; 64 (2); 69; 73; 75; 83; 87; 88; 105; 115; 131; 139; 173; 200; 223; 232; 294; 326; 603; and 1799.
Total catch: 6814 individuals representing 197 species.

An example is given in table 1 of the numbers of species with different numbers of individuals in a sample of Macro-Lepidoptera taken in a light trap at Rothamsted Experimental Station, Harpenden, about 25 miles north of London. The distribution is also shown diagrammatically on figure 1.

It will be seen that the 94 least common species (those with one to five individuals each) provide only 220 individuals out of a total of 6814. That is to say that 48 per cent of the species provide only 3.2 per cent of the individuals. At the other end, the single most abundant species provides 26.4 per cent of the total population, and the six most abundant species (three per cent of the total) provide 50 per cent of the individuals.

In the Audubon bird counts in the United States in 1954 one species accounted for 45 per cent of the individuals out of a total of 17 million, while in a total of seven year-counts one species accounted for 35.5 per cent of the total (see below). In dozens of other samples from wild populations a similar pattern is found.

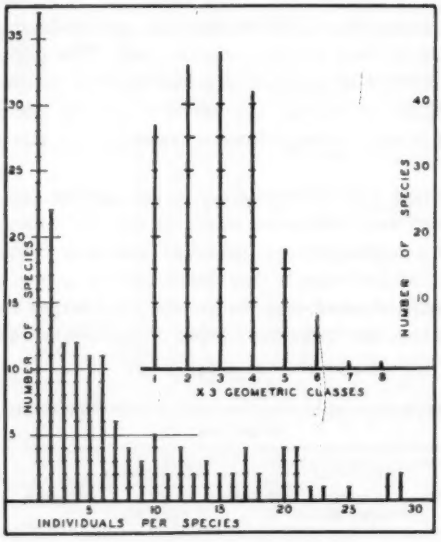

FIGURE 1. The frequency distribution of species with different numbers of individuals in a sample of 6814 individuals of Macro-Lepidoptera belonging to 197 species, caught in a light trap at Harpenden, England, in 1935 (for full data see tables 1 and 2). In the lower diagram the classes of individuals per species are on an arithmetic scale, in the upper diagram in a geometric scale.

LOGARITHMIC SERIES

In 1943 it was suggested (Fisher, Corbet and Williams, 1943) that the pattern of distribution closely resembled the mathematical "logarithmic series," which has the form

$$n_1 : nx/2 : nx^2/3;\ nx^3/4 \ldots \text{etc.}$$

or

$$\alpha x : \alpha x^2/2;\ \alpha x^3/3;\ \alpha x^4/4 \ldots \text{etc.}$$

where the successive terms are the number of species represented by one, two, three, etc. individuals.

The total number of species (S) is given by $S = \alpha(-\log \overline{1-x})$ and the total number of individuals (N) by

$$N = \frac{\alpha x}{1-x}$$

There are only two parameters, and so the form of the distribution can be calculated if N and S are known. "α" is a measure of the diversity of the sample; and "x" is dependent on the size of the sample, being similar for all samples with an equal average number of insects per group (N/S).

In the example given in table 1, the value calculated for the number of species with one individual from the total individuals and total species, on the assumption of the logarithmic series, is 37.94, while the observed value was 37.

It is important at this point to note that the data available for study are nearly always in the form of more or less random samples of the population; and it is to these samples that the mathematical order has been found to apply. But since it is impossible, by the process of sampling, to make order out of chaos, it is clear that the demonstration of a mathematical order in a sample must be an indication of some similar or related mathematical order in the population that is being sampled.

It can be shown mathematically that if a population of units classified into groups (for example, individuals and species), which has the frequency distribution of a logarithmic series, is randomly sampled by units, then the sample itself will be also distributed in a logarithmic series; with the same value of α, and a smaller value of x. There is therefore an *a priori* reason for considering that if the sample is of the form of a log-series, it is likely that the population was also of this form.

A number of papers have been published on the application of the log-series to various samples from populations of insects, birds, fishes, and even plants (see Williams, 1944, 1950, 1953), but it became evident that in very large samples the fit was not so close as in the smaller samples. It might be that the log-series was only an approximation to the real distribution.

THE LOG-NORMAL DISTRIBUTION

In 1948 Preston suggested that the frequency might be of the form of a log-normal distribution, or possibly a truncate log-normal, that is to say, a normal distribution when the scale for numbers of individuals per species is classified geometrically and not arithmetically. This series is found, in many cases, to fit the observed values better than the log-series (see Williams, 1953; Preston, 1948 and 1958).

When the data already given in table 1 are classified on a $\times 3$ geometrical scale (see Williams, 1953, p. 17) for the number of individuals per species, we get the results shown in table 2 and figure 1B. The distribution in table 2 shows a moderately good fit to a truncate log-normal.

TABLE 2

Class	Individuals per species	No. of species
1	1	37
2	2–4	46
3	5–13	48
4	14–40	37
5	41–121	19
6	122–364	8
7	365–1093	1
8	1094–3280	1

The log-normal distribution is more difficult to treat mathematically, as it has three parameters, and so there is no single distribution for a given number of individuals and species. A third constant is necessary which can be some measure of the spread—or standard deviation—of the distribution. This can often be obtained approximately by a graphical examination of the available data.

TABLE 3

Class	Individuals per species	No. of species
1	1–10	7
2	10–100	9
3	100–1,000	22
4	1,000–10,000	42
5	10,000–100,000	32
6	100,000–1 million	16
7	1 million–10 million	12
8	just over 10 million	2
	Total individuals: about 63 million	Total species: 144

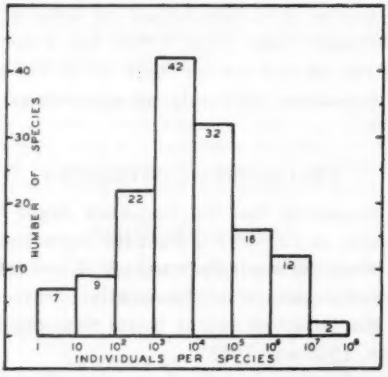

FIGURE 2. Frequency distribution of species with different numbers of individuals for estimated population of 144 British land-nesting birds, representing a total of about 63 million individuals. The number of individuals per species in × 10 classes (for data see table 3).

An example of a close fit to a log-normal distribution is shown in table 3 and figure 2. It is an estimate, made by James Fisher, (partly from censuses) of the number of species of land-nesting birds in England and Wales with different numbers of individuals, the number of individuals per species being classed in geometric $\times$ 10 classes. For fuller details see Williams (1953) p. 29.

This gives a very good log-normal distribution with a median at $10^{3.9} = e^{9}$ = 8000, and a standard deviation of $10^{1.4} = e^{3.2}$.

Preston (1958) gives frequency distributions for some Audubon bird counts in the United States which fit closely to slightly truncate log-normals. The count for 1955 included 24.7 million birds of 487 species with an S.D. of $e^{3.24}$. The total of four years' counts (1954–1957) gave 83.4 million birds in 518 species, with an S.D. of approximately $e^{4.15}$.

INSECT ABUNDANCE

Since so many random samples, taken by different methods, from different groups of insects, and in different parts of the world, have indicated a regular mathematical pattern in the number of species with different numbers of individuals, it is interesting to speculate what might be the frequency distribution pattern of all the insects in the world at any given moment—and what one might infer from it about the absolute abundance of species.

For such an inquiry it is necessary to have estimates, based on some form of reason, for two or three constants, of which perhaps the total number of species and the total number of individuals are the most possible to obtain. If we make the provisional assumption that the frequency distribution is a logarithmic series, these two are sufficient to define the series and all its properties. If we postulate a log-normal distribution, a third constant or parameter is needed, which could be the measure of the spread of the distribution round the mean—in other words, the estimate of the standard deviation.

The total number of species of insects at present known is larger than that of any other group of animals. In fact, in the British Isles, the number of known species is already over 20,000 and is greater than that of all the other groups of animals and plants added together. Various estimates of the number of already described species of the insects in the world have put the number somewhere near one million, though Metcalf (1940) suggests one and a half million. New species are being described at the rate of about 10,000 a year. But in view of the numerical predominance of rare or very rare species in all examined random samples we must assume that, for the world as a whole, most of the extreme rarities are still unknown. My own opinion is that most certainly less than half the existing species are as yet described, and that the proportion may be as little as a third or even one fifth. I suggest therefore that we take as a low estimate two million, and as a high estimate five million species of insects as a world total, with three million as a likely approximation.

To estimate the number of individual insects existing in the world at any given moment is an even more questionable occupation. In a series of careful extraction of insects from the soil at the Rothamsted Experimental Station in southeast England, the numbers in the top few inches of soil gradually rose, with improvements in technique, from 20 million to about 200 to 400 million insects per acre. One acre contains approximately 40 million square centimeters, so the indicated population was about 5 to 10 insects per square centimeter of surface. We do not yet have sufficient information about the population in tropical countries, or in hot and cold deserts; nor have we any accurate measurements of the number of insects that are normally above ground. Many places, however, must be more prolific than southeast England, and many less so. Since the land surface of the earth is not far from 50 million square miles, the number of square centimeters is of the order of 1.3×10^{18}. On our British estimates we would therefore suggest about 8×10^{18} insects for the total. In view, however, of the great expanse of cold areas such as Greenland and the sub-arctic areas, this is possibly an over-estimate. It would seem that the total is unlikely to be less than 10^{17} or more than 10^{19}. Let us therefore take 10^{18} as a working hypothesis.

If we take the 10^{18} estimate of individuals and two, three or five million species, we get an arithmetic mean number of individuals per species as one half, one third or one fifth of a million million respectively. It must be remembered that, because of the almost certain geometric scale of abundance, this *does not* mean that half the species have more and half less than this number. The median is not at the arithmetic mean, but probably at the geometric mean (see below) and thus much lower.

Is this estimate for the arithmetic mean absurd? I have myself seen a single swarm of locusts which I calculated to contain about ten thousand million (10^{10}) individuals and, as swarms go, this was not considered an unusually large one. During a flight of Painted Lady Butterflies in California in 1929, McGregor (1924) estimated that there were about three thousand million butterflies concerned. But both of these are comparatively large insects. It is probably amongst the smaller species, such as Collembola and aquatic insects such as midges, may-flies and mosquitoes that the really large numbers occur. It would seem not unlikely that the number of mosquitoes in some cold-temperate or sub-arctic regions must be measured in millions of millions. Large populations are also likely to be found in species with very wide distributions throughout the world.

When we try to apply mathematical methods we must make an assumption as to the probable form of the distribution. Two such distributions—the logarithmic series and the log normal—have, as previously stated, been found to be possible explanations of random samples of insects.

THE LOGARITHMIC SERIES

For the logarithmic series, if we know the total number of species and individuals, the rest of the properties of the distribution can be calculated: of

particular interest to us would be the number of species with one individual, and the median level of abundance.

Figure 1 shows, for very high values of N and S, the relation between the number of individuals, the number of species, and the number of species with one individual, which in the case of these high numbers is practically identical with the Diversity "α." The values suggested above for the total insect population are within the dotted rectangle. It will be seen that with 10^{18} individuals and three million species, there should be approximately 100,000 species represented at any one moment by a single individual

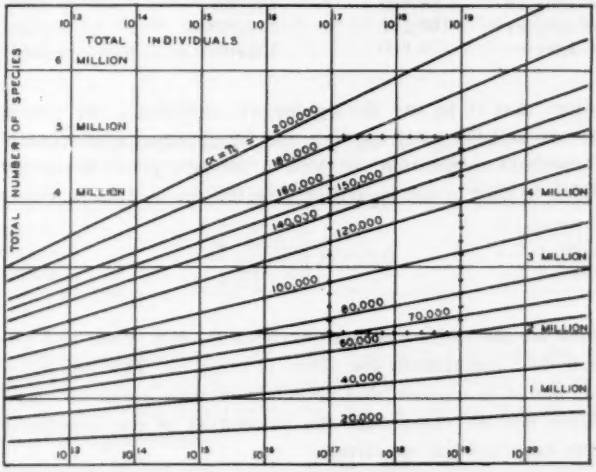

FIGURE 3. The relation between the number of individuals (on a logarithmic scale) and the number of species (on an arithmetic scale) for large populations, based on the assumption of a log series distribution. Each diagonal line represents a different Index of Diversity which, for these very large samples, is practically identical with the number of species represented by a single individual.

only—presumably species which are either incipient or just dying out. This is 3.3 per cent of the total assumed number of species. If we take the lower number of two million species, those represented by one individual are approximately 65,900. If we take the upper limit of five million species, those with a single individual should be 170,000. In each case these constitute almost exactly the same proportion of the total.

When the average number of insects per species is high, the value of "x" in the logarithmic series comes very close to unity, and the series comes —at least in its earlier terms—very close to the hyperbolic series: 1, ½, ⅓, ¼, etc. Where $N = 10^{18}$ and S = 3 million, the calculated value of x = 0.999,999,999,999,8998, and it is possible to assume that at least the first half of the series will be extremely close to a hyperbola. Using, for the terms above 1000, the approximation that the sum of the hyperbolic or harmonic series up to the "r" term = $n_1(\log_e r + 0.5772)$ we get the propor-

tion of species with different numbers of individuals as shown in table 4, which also shows the values for two and five million total species.

From the formula used, it follows that each increase in a power of ten of the individuals per species adds to the total species up to that level (except in the lower values) a number equal to $n_1 \log_e 10 = 2.3026\ n_1$. For the three values of n_1 corresponding to the three levels of assumed total population, the addition for each $\times 10$ class runs as follows:

	n_1	Increase for class	Per cent of total
2 million species	65,900	151,741	7.59
3 million species	100,200	230,700	7.69
5 million species	170,000	391,442	7.83

The median, that is to say the number of individuals per species above which there are half the total species, can be obtained approximately either from the hyperbola summation, or from a formula given to me by Dr. P. Grundy; which is that in the logarithmic series the median is approximately

$$0.81524 \times \frac{0.56146}{\sqrt{1-x}}$$

The values for the median by both methods for two, three and five million species with 10^{18} individuals are given in table 5. The two estimates are very close, but it is curious that Grundy's formula gives in each case a slightly lower median value than the summation of the hyperbolic series. I would have expected the opposite.

TABLE 4

Species with up to this number of individuals per species	Total species out of: 2 million		3 million		5 million	
	up to the level of the first column					
	Number	Per cent	Number	Per cent	Number	Per cent
1	65,900	3.3	100,200	3.3	170,000	3.4
10	193,100	9.75	293,600	9.8	498,100	10.0
100	342,000	17.1	520,000	17.3	882,300	17.6
1,000	493,300	24.7	750,000	25.0	1,272,000	25.5
10,000	645,000	32.2	980,700	32.3	1,663,900	33.3
100,000	796,700	39.8	1,211,400	40.4	2,055,300	41.1
1 million	948,500	47.4	1,442,200	48.1	2,446,800	48.9
10 million	1,100,200	55.0	1,672,900	55.7	2,838,200	56.8

The percentage of the total species at different levels of abundance is almost the same irrespective of the total assumed number of species. It will be seen from table 4 that there is only a very slight increase as the total

number of species is increased. In all three cases the number of species which should be represented by a single individual is very close to 3.3 per cent or one in 30 of the total species.

This estimate of over three per cent of the total species being at any moment so excessively rare as to have only a single individual per species, and almost 10 per cent with not more than 10 individuals, seems to be very high, but it must be remembered that the proportion of the total *individuals* in such species is microscopic. Out of the assumed 10^{18} individuals one would have to examine a random sample of about fifteen million million insects before having a level chance to get a single individual of one such species. Even for those species with up to 10 individuals one would have to collect about 1.5 million million.

TABLE 5

Median number of individuals per species

Species	From hyperbola	Grundy's formula
2 million	2,187,000	2,185,000
3 million	1,778,000	1,773,000
5 million	1,367,000	1,359,000

I asked Professor Haldane some years ago if in his view the number of about 66,000 incipient or dying species (on the assumption of a total of two million) at any one time was excessive, but he replied that he might have expected a still higher number.

The distribution in the log. series at the upper limits of population, that is, the number of species with very high numbers of individuals, is a complex mathematical problem which requires further investigation.

THE LOG-NORMAL DISTRIBUTION

The problem of fitting a log-normal distribution to all the insects of the world is an even more doubtful proceeding than fitting a logarithmic series but is, I think, more likely to be correct. To define the log-normal we must have the three constants or parameters, which may be (from our point of view) any three of the following:

(1) N = the total number of individuals.
(2) S = the total number of species.
(3) σ = the standard deviation of the distribution (= e^{σ} on an arithmetic scale).
(4) a = the position of the median on the logarithmic scale, or, on an arithmetic scale, the geometric mean number of individuals per species.

The interrelation of these is given by

$$N = S\, a\, e^{\frac{1}{2}\sigma^2}$$

or alternatively the arithmetic mean number of individuals per species

$$N/S = ae^{\frac{1}{2}\sigma^2}$$

If we make the same assumption as before about the possible numbers of individuals and species, we get for each pair of these a number of possible alternative pairs of values for a and σ.

For example, if we assume that $N = 10^{18}$ and $S = 3 \times 10^6$, then the arithmetic mean number of individuals per species = 3.3×10^{11}, or one third of a million million.

If we suggest that $\sigma = 3$, it follows that $e^{\frac{1}{2}\sigma^2} = 90$ and therefore the median of the log-normal distribution would be at 3.67×10^9 ($= e^{22}$). In other words, half of the species in the world should contain more than this number of individuals, and half should contain less.

TABLE 6

Possible values of S, σ, and a for 10^{18} individuals

Values of S →		2 million	3 million	5 million
Values of N/S →		5×10^{11}	3.3×10^{11}	2×10^{11}
σ	Values of $e^{\frac{1}{2}\sigma^2}$	Values of median (a) corresponding to above values of N and S		
2	7.4	$6.75 \times 10^{10} = e^{24.9}$	$4.46 \times 10^{10} = e^{24.5}$	$2.7 \times 10^{10} = e^{24.0}$
3	90.0	$5.5 \times 10^9 = e^{22.4}$	$3.67 \times 10^9 = e^{22.0}$	$2.2 \times 10^9 = e^{21.5}$
4	2,981	$1.68 \times 10^8 = e^{18.9}$	$1.17 \times 10^8 = e^{18.7}$	$6.7 \times 10^7 = e^{18}$
5	268,000	$1.88 \times 10^7 = e^{16.7}$	$1.23 \times 10^7 = e^{16.3}$	$7.5 \times 10^6 = e^{15.8}$

To obtain the means for 10^{17} and 10^{19} individuals, the values given above are divided or multiplied by 10.

Table 6 shows a number of alternative values for N, s, a, and σ which are likely to be somewhere within reason. Figure 4 shows the same data set out diagrammatically for S = two, three, or five million species, $N = 10^{18}$ individuals, and standard deviations of e^2, e^3, e^4 and e^5, together with the number of species in each S.D. range from five S.D. above and to five S.D. below the median. The total assumed population (10^{18}) is shown as a heavy line on the right of the diagram.

Taking the lower value of S.D. = e^2 it would appear that, with a median at 4.5×10^{10} individuals per species, less than one out of the three million species could be represented by fewer than about two million individuals. This seems to me much too high a lower limit. With S.D. = e^3 there would be less than one species in three million with fewer than 10,000 individuals. This is still high. If the number of assumed species is increased to five million or reduced to two million, there is not a great alteration in this limit.

If the total number of individuals is assumed to be greater at 10^{19}, or less at 10^{7}, then the lower limits are multiplied or divided by 10, but still appear to be high, even when divided.

If we go to the higher estimate of S.D. = e^5 we get a possible upper limit of individuals per species exceeding the total population. This may be due to errors of approximation with the very large numbers involved. At the lower limit the distribution is slightly truncate with about 3000 species (0.1 per cent) out of an assumed three million below Preston's "Veil line" of one individual per species. Fractional values below unity can exist in mathematical averages, but not in reality.

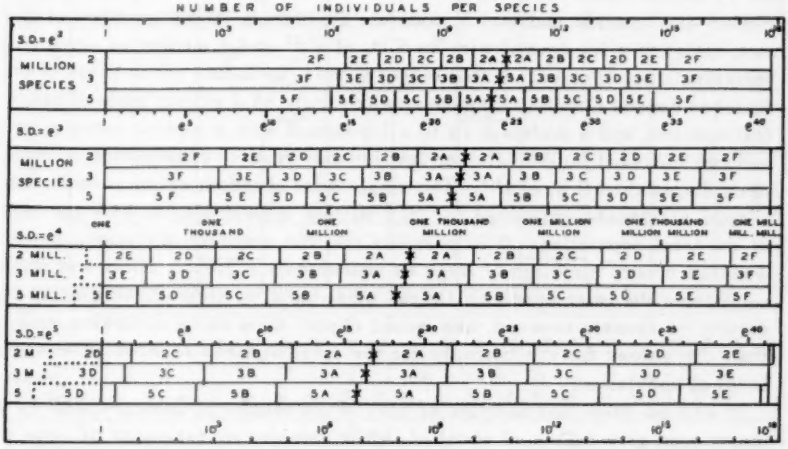

FIGURE 4. Possible frequency distributions of the total insect population of the world, based on the assumption of a log-normal distribution. Values are shown for an assumed population of 10^{18} individuals and for S.D.'s from e^2 to e^5, and total number of species at two, three, and five million.

	A	B	C	D	E	F
2:	682,600	271,800	42,800	2638	64.4	0.6
3:	1,023,900	407,700	64,200	3957	93.6	0.9
5:	1,706,500	679,500	107,000	6595	156.0	1.5

On the whole, if the log-normal distribution is to apply it would seem that an estimate of S.D. = e^4 is the most reasonable. This gives, for three million species, about 60 species with less than 10 individuals, and at the other end perhaps one species with 10^{17} individuals or about 10 per cent of the total population.

Some confirmation of this suggested value for the S.D. may be obtained from known large samples, censuses, and estimates of animal populations. A sample of about 32,000 Lepidoptera taken in a light trap in southeast England during eight years, and including 285 species (Williams, 1953, p. 23) gave, when fitted to a log-normal distribution, an S.D. of approximately $e^{2.2}$. Another sample of about 59,000 moths taken in the same locality in

two mercury-vapor light traps in two years, gave an S.D. of e^2 (Hosni, unpublished data). The latter sample had more individuals, but was taken in a much shorter period, and so might be expected to be less diversified.

An estimate of the total land-nesting bird population of England and Wales, made by James Fisher, gave approximately 63 million birds belonging to 143 species and fitted very closely to a log-normal distribution, with an S.D. of approximately $e^{3.2}$ (Williams, 1953, p. 29). An estimate of the whole of the bird population of the Nearctic area (Preston, 1948) at 10^{10} individuals (roughly one pair per acre) and about 606 species, fitted a log-normal with an S.D. of $e^{3.74}$. Another calculation by Preston (1958, p. 623) for 25 million birds observed in the Audubon Christmas Survey in 1955, included 487 species and was a moderate fit (except in the numbers of the rare birds) to a log-normal with an S.D. of $e^{3.25}$ and a median at about 250 individuals per species. A still bigger sample, including seven years of the Audubon census (1949–1955) gave approximately 83.4 million birds of nearly 600 species, and a moderate fit to a log-normal with a median between one and two thousand individuals per species and (from an examination of Preston's diagram) an S.D. of $e^{4.1}$. The single most abundant species—the red-winged blackbird—accounted for 29.6 million individuals, or 35.5 per cent of the total population. It is possible that the standard deviations in the Audubon counts are slightly large as undoubtedly a greater effort was made to observe the rarer species. On the other hand, in view of the greater diversity of country covered, one would expect them to be somewhat larger than the values for the estimate for the English birds of about the same level of population.

It will be seen that samples of tens of thousands of insects taken in a small area give S.D.'s of about e^2, while samples or estimates of tens of millions of birds over large areas give values of about e^4. There may of course be some basic difference between the frequency distributions of insects and of birds, but the differences that we find are what one might expect from increasing size of sample and diversity of habitat alone. It thus seems that an estimate of e^4 or over is not unlikely to be reasonable for the whole insect population.

On these assumptions, figure 5 shows the pattern of distribution of the abundance of the insect species of the world. The background curve and histogram is a log-normal showing the limits of successive classes of one S.D. above and below the median, and also the percentage of the total species in each class. Below this are the expected numbers out of three million species, and at the bottom the scales, in powers of 10, and powers of e, for 10^{18} and 10^{19} total individuals.

With 10^{18} individuals, about two thirds of the total species will lie between $10^{6.3}$ (– 1 S.D.) and $10^{9.8}$ (+ 1 S.D.) or approximately three million and six thousand million individuals per species. Just over one quarter of the total species will be divided equally between the less abundant class $10^{4.6}$–$10^{6.3}$, and the more abundant class $10^{9.8}$ and $10^{11.5}$, (that is, 40,000 to three mil-

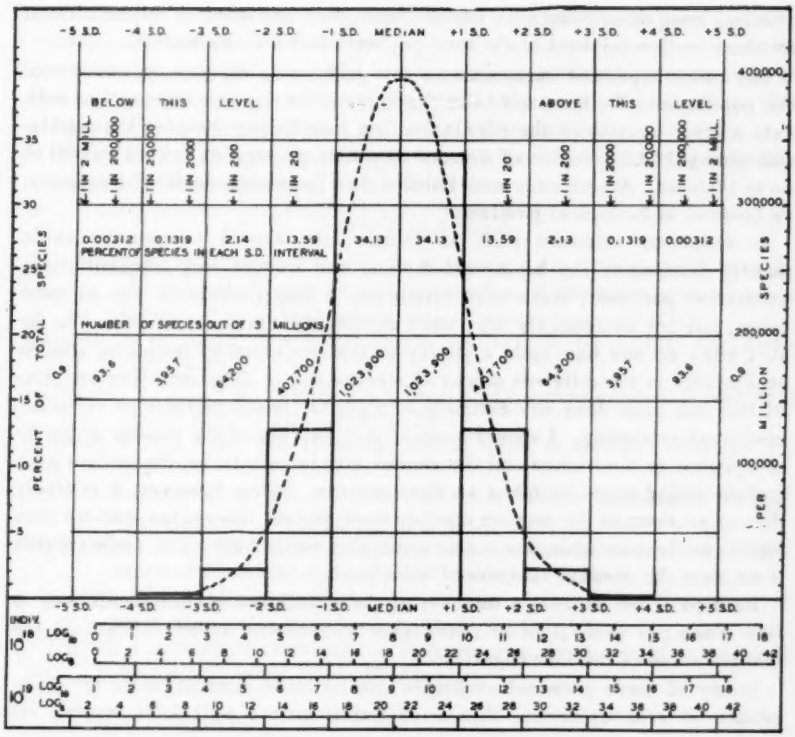

FIGURE 5. Diagrammatic representation of the possible frequency distribution of the insects of the world based on the assumption of the log-normal distribution, with an S.D. of e^4, three million species, and scales at the bottom for 10^{18} or 10^{19} total individuals. The total species are divided into classes equal to one S.D. departure up and down from the median.

lion, and six thousand million to 300 thousand million individuals per species).

One in 20 thousand species will have less than 20 individuals at the bottom end, and at the top end one in 20 thousand species will have more than $10^{14.8}$ or 700 million million individuals. And finally about one species in two million might be expected to have over $10^{16.5}$ or 30 thousand million million individuals.

This suggests that out of three million species, one or two could contain above three per cent of the population, and with normal variation the figure might well be five per cent or above. In view of the great evolutionary diversity of the world fauna, as compared with that of a smaller area, one would not expect any one species to dominate to the extent of 20–40 per cent, as found in some of the examples already given. On the whole the con-

clusion, from these numerous assumptions, that one species might account for three to five per cent of the total population is not impossible.

One other aspect of importance in this problem is the rate of turnover of the population. We have used the expression "at any one moment" to indicate a cross-section of the population, but how frequently does the population change? Life cycles of insects vary from perhaps as low as one week up to 17 years. Are we once more faced with a geometric scale of frequency, so common in biological problems?

In temperate climates such as Britain, the annual temperature cycle largely determines the biological rhythm, and we get very frequently one generation per year; much less frequently a larger cycle of two or more years, but not uncommonly two, three or more generations per year. So far as I know no one has made a survey of the frequency of longer or shorter generations in the different orders of insects and in different climates. Until this has been done any estimate is a guess, based perhaps on some unconscious reasoning. I should suggest that, in view of the greater diversity of species in the tropics and the shorter generations there, the median generation period might be taken as three months. Since, however, it is likely that on an average the smaller species have shorter life cycles, and are also likely to be more abundant, some correction would have to be made for this if we want the average turnover of individuals.

Perhaps if we suggest a turnover of the population of individuals four or five times per year, it is as reasonable an estimate as we can get in the present stage of the investigation.

If any of these personal estimates can be made more accurate by an increase in real evidence, this speculative article will have served its purpose.

SUMMARY

Recent work on the relative abundance of species in samples taken from animal and plant populations in the field shows that there is nearly always evidence of a regular pattern of frequency distribution. This has usually been expressed in terms of the logarithmic series (which fit best to smaller samples) or the log-normal (which fits better to larger samples). The present article is a speculative attempt to extend these two series to the insect population of the whole world. It is suggested as a working basis that at any moment the insect population of the world is 10^{18} individuals and that there are perhaps as many as three million species. On these assumptions the log-series indicates a very large number of rare species, with the median number of individuals per species just under two millions. With the log-normal it is necessary to make a further assumption of a value for the standard deviation of the distribution and from evidence of large samples it is suggested that this might be approximately e^4. In this case the median number of individuals per species will be (for 10^{18} individuals and 3×10^6 species) approximately 1.7×10^6.

The probable number of individuals in the most abundant species is also considered. For the log-series there is not yet any reliable formula which can be extrapolated so far. With the log-normal, it would seem that one or two species out of three million might contain three per cent or perhaps five per cent of the total population, or $3 - 5 \times 10^{16}$ individuals.

The object of this paper is not to present finality but to encourage discussion and the production of new evidence.

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ON THE EMERGENCE OF INTRASPECIFIC DIFFERENCES IN THE PROTEIN ANTIGENS OF HUMAN BEINGS

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It has long been known from the serological studies of Uhlenhuth (1901a, b), Nuttal (1904), Boyden (1926), Wolfe (1935) and many others that proteins change in antigenic specificity as living organisms take divergent evolutionary paths. This change, accumulating with the passage of time, is for the systematic serologists a very gradual one. Thus the chances of detecting differences in protein specificity between human beings may have appeared at best quite slim, and leads such as those of Cumley and Irwin (1943) pointing to an intraspecific variation of protein antigens distinct from that of the blood isoagglutinogens were not immediately pursued. However, the discovery by Pauling et al. (1949) of a molecular abberation in the major hemoglobin of patients with sickle cell anemia clearly established that evolutionary changes in protein specificity could be detected at the species level.

A number of examples of genetic variants in mammalian proteins have now been brought to light. On the whole, this has been done by the application of physico-chemical techniques (see Allison, 1959, for a review of pertinent examples). Yet the immunological approach has also been successfully employed. Goodman and Campbell (1953) found that a small but significant antigenic difference between sickle cell anemia hemoglobin and normal adult hemoglobin could be detected with chicken antiserum. Grubb (1956, 1957), Oudin (1956), Goodman et al. (1958a, 1959a), Dray and Young (1958, 1959) and Dubiski et al. (1959) have each found examples of intraspecific variation in the serological specificity of serum protein components. Certain of these, such as Grubb's human gamma globulin groups and the rabbit gamma globulin groups (Dray and Young, 1959, and Dubiski et al., 1959) have been shown to have a genetic basis. No doubt other examples of genetic variants will be found. Such protein types, in contrast to the isoagglutinogens, are apt to show those attributes of Loeb's "organismal differentials" (Loeb, 1945) indicative of the individualization of organisms, because their unique structural configurations which are held by only a fraction of the population within the species probably fail to occur outside the species. In this sense, these serum proteins as antigens may prove to be analogous to the histocompatibility antigens of transplantation immunity.

The purpose of this communication is to present (1) some data, gathered with chicken antisera, illustrative of this type of serum antigen and (2) a hypothesis concerning the effects of protein antigenicity on the evolution of human beings. The data deal with an age-dependent, phenotypic variation

between human beings in their gamma$_2$ globulin and represent an extension of the original observations (Goodman et al., 1958a; 1959a), in that an analysis of twins and their parents for the presence or absence of certain of the gamma$_2$ components has been initiated.

EXPERIMENTAL

The key immunizing antigen was a water soluble gamma$_2$ globulin (ps γ_2) isolated from Cohn FrII of pooled human plasma. Several lots of FrII and protein fractions from individual human subjects were also employed. Dr. John K. Inman prepared the ps γ_2, generously making it available for these studies, and the Blood Products Section of the Michigan Department of Health, the American National Red Cross, and the Sharpe & Dohme Research Laboratories provided the lots of FrII. The antisera were readily produced in year-old hens by the single injection procedures reported in previous publications (Goodman et al., 1957a; 1957b; 1958a). A uniformly successful procedure, not previously reported, was to inject a hen with 25 mg. by the intracardiac route and also with 75 mg. mixed with alumina cream adjuvant by the intramuscular route into several spots and then bleed the hen ten days later. The antisera were reacted against human serum samples by a starch gel immunoelectrophoretic method after Poulik (1959), and a number of these antisera detected more than one gamma$_2$ globulin component in some of the human sera but not in others.

After initial experience was gained, newly produced antisera were routinely screened against two pools of human sera, one yielding positive reactions with previous reagents and the other yielding negative reactions with many of these previous reagents. A 0.1 ml. of each pool would be incorporated in a starch paste, inserted at a line of origin in a starch gel block, and then separated into its various serum protein components by the starch gel electrophoretic method of Smithies (1955). Portions of the starch gel block and starch paste containing the gamma globulin proteins would then be placed at the bottom of an optically clear (agar precipitin analysis) chamber, 0.75 per cent agar would be poured to a 1 cm. depth above the starch gel, and then the antiserum reagent would be layered above the hardened agar. The chamber would be sealed and observed usually for a 12 day period for the development of antibody-antigen precipitin reactions within the agar. The gamma globulin portions of both the "positive" and "negative" pools could be placed in the same chamber to permit simultaneous comparison with each antiserum being screened.

Figure 1 reproduces the reactions (developed in three chambers) of an anti-ps γ_2 serum, C-42, which detected in the "positive" pool an additional gamma$_2$ globulin component. It can be seen that as C-42 was absorbed with increasing amounts of the "negative" pool the major gamma globulin precipitin arc disappeared, but the minor gamma$_2$ precipitin arc still remained. With a number of the antisera this major gamma globulin precipitin arc, one encompassing the zones of slow and fast gamma mobility, cloaked the precipitin reaction centered in the zone of slow gamma mobility. In such in-

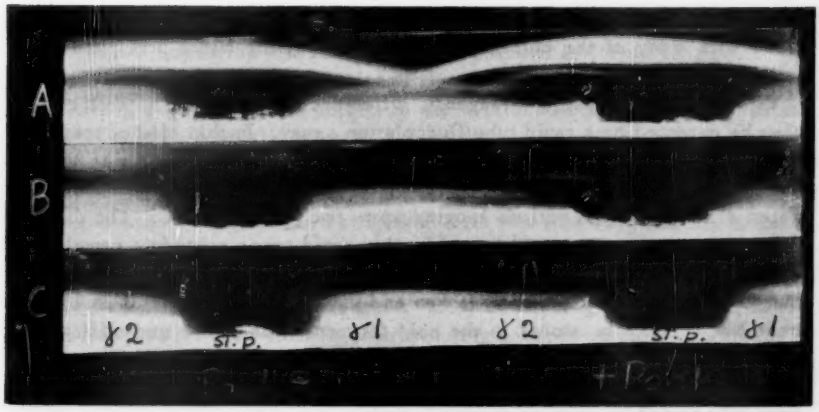

FIGURE 1. The gamma globulin reactions in starch gel immunoelectrophoresis afforded by the "negative" human serum pool and the "positive" human serum pool with a chicken antiserum, C-42, to ps γ_2.

A. The antiserum was unabsorbed. Note the strong precipitin reaction with the predominant gamma globulin antigen in both pools and the additional reaction for a gamma$_2$ globulin antigen in the positive pool.

B. The antiserum was absorbed per ml. with an aliquot of the negative pool estimated to contain 194 mcg. of gamma globulin. Note that there were still some antibodies left for the predominant gamma globulin antigen.

C. The antiserum was absorbed per ml. with an aliquot of the negative pool estimated to contain 388 mcg. of gamma globulin. Note that in this large excess of antigen the absorbed antiserum still yielded a good reaction for the additional gamma$_2$ globulin antigen.

The nonspecific lateral band that developed in the agar near the surface of the starch gel block could be so recognized and thus discounted in assessing the specific antibody-antigen reactions.

γ_2 = The gamma$_2$ globulin portion of the starch gel block.
st.p = Starch paste, in which the serum sample was originally inserted.
γ_1 = The gamma$_1$ globulin portion of the starch gel block.

stances, a partial absorption of an antiserum with the "negative" pool fragmented the gamma globulin precipitin arc, revealing the minor gamma$_2$ reaction as a distinct arc beneath the major, but now diffuse, arc. (The nonspecific precipitate line that formed laterally above the starch gel block could readily be ruled out in making these interpretations.) Then, upon continuing the absorption of the antiserum with increasing quantities of the antigen, a point was reached as in the case of C-42 where the major gamma globulin reaction disappeared but the minor gamma$_2$ reaction was still exhibited. Doubling or quadrupling the quantity of antigen needed to reach in an absorption an excess of unprecipitated human gamma globulin molecules did not remove from the antiserum all the antibodies reacting with a fraction of the gamma$_2$ globulin molecules of some human sera. Generally, however, a smaller number of the human sera would then yield this minor reaction as compared to those yielding it in the case of the partially absorbed antiserum. The innovation, introduced by Goodman et al. (1958b), of incorporating 13 per cent NaCl in the agar proved particularly important in demonstrating

these minor gamma$_2$ reactions. At 1 per cent NaCl and even at 8 per cent NaCl with many of the chicken antisera reagents the minor precipitin line failed to form.

With the heavily absorbed reagents, differences between human sera could also be detected by a rapid tube flocculation assay. In this kind of testing, a 0.1 ml. of a 1/10 dilution of human serum in 13 per cent NaCl was mixed with 0.2 ml. of reagent, and the presence or absence of precipitation was noted after incubation periods ranging up to two hours at 38°C. The differences between human sera detected by this rapid assay procedure correlated with those observed by the immunoelectrophoretic analyses. The samples of human sera could be repeatedly frozen and thawed or, after dilution in 13 per cent NaCl, could be stored in the cold for months at a time and still yield reproducible reactions for the minor gamma$_2$ components. Similar reactions were also afforded by repeat samples drawn from the same subject over a year long period.

An initial effort was made to establish if there was a genetic basis to the presence or absence in human subjects of the gamma$_2$ globulin molecules containing the unique antigenic configurations. Sera from the members of 26 families were conveniently obtained with the kind cooperation of Dr. Benjamin Schwimmer, Director of the Social Hygiene Clinic of the City of Detroit, Department of Health. Upon analyzing these sera with a reagent prepared from pooled anti-ps γ_2 serum, a striking age effect was observed. Sixty-eight per cent of the parents (age 19-52 years) yielded clear positive reactions for the minor gamma$_2$ component, whereas only 19 per cent of the prepuberty children did. Since of the total number of children (88 in all) two-thirds fell in the prepuberty bracket (0-9 years), the age effect obscured any heredo-familial factor that might be operative in the appearance of the minor gamma$_2$ globulin antigen.

Though the children on the whole were free of pathologies, their parents were equally split between those carrying venereal disease (mainly in the latent form) and those who were normal. Both of these adult groups yielded the same incidence of positive reactions for the minor gamma$_2$ antigen, thus ruling pathology out as a factor in accounting for the results. Similarly, the quantitative gamma globulin content of the serum samples could not have been a primary factor behind the appearance of the minor antigen, for when those samples which had gamma globulin levels close to a mean of 13 mg./ml. were compared (29 parents to 39 prepuberty children) the same striking age effect was seen. Subsequently, several series from the Social Hygiene Clinic of prepuberty and postpuberty subjects (comparable in numbers to the family study) have been screened with new chicken antiserum reagents, and again minor gamma$_2$ components were detected much more frequently in the postpuberty group. For example, in one series of 33 postpuberty subjects (14-38 years) and 52 prepuberty subjects (0.2-9 years) 58 per cent in the older group yielded positive reactions as compared to only 13 per cent in the younger group. In another series of 60 postpuberty subjects (14-45 years) and 45 prepuberty subjects (0.2-9 years) 72 per cent in the

older group as compared to 22 per cent in the younger group yielded positive reactions on employing this time a different chicken antiserum reagent.

In passing, it should be mentioned that certain chicken antisera yielded the second gamma$_2$ globulin precipitin arc with almost all samples of human sera. However, generally after sufficient absorption with a suitable pool of prepuberty sera, these antisera did discriminate between different samples of human sera. Nor was the same set of unique antigenic configurations always detected by the absorbed reagents. With two such reagents human sera fell into four groups; (a) sera reacting with both reagents, (b) sera reacting only with one reagent, (c) sera reacting only with the other reagent, and (d) sera reacting with neither reagent. Many of the reagents, however, have tended to parallel each other in discriminating between human serum types.[1]

A study of the reactions of such reagents with the sera of young adult twin pairs (18-22 years) and their parents is now under way. The majority of the subjects are being contacted from the list of monozygotic and dizygotic twins intensively studied at the Department of Human Genetics, Uni-

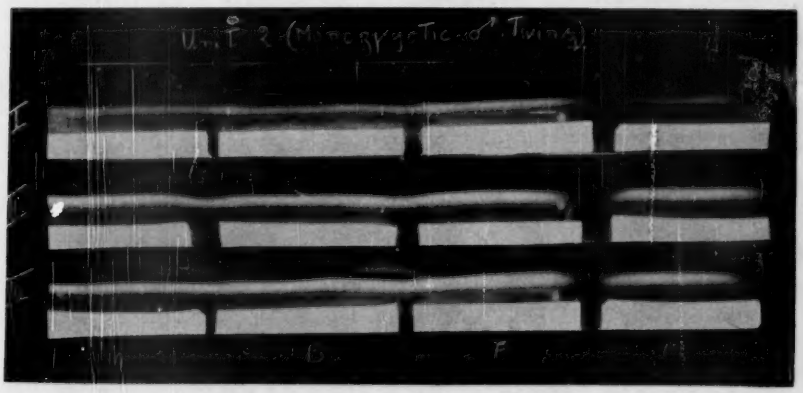

FIGURE 2. The agar precipitin analyses of an identical twin pair and their parents upon isolating from each serum sample the gamma globulin of slow (γ_2) mobility by starch gel electrophoresis. A = One member of the twin pair; + reaction. B = The other member of the twin pair; + reaction. F = The father; − reaction. M = The mother; + reaction.

I. A chicken anti-ps γ_2 serum (C-90) absorbed per ml. with an aliquot of pooled "negative" human serum containing 230 mcg. of gamma globulin. The reagent contains unprecipitated human gamma globulin, but still detects as seen a component of γ_2 mobility in some human sera.

II. A chicken antiserum (C-330) produced to a globulin fraction of a human subject and absorbed per ml. with an aliquot of the "negative" pool containing 130 mcg. of gamma globulin. The reagent contains unprecipitated human gamma globulin.

III. C-330 absorbed per ml. with only 65 mcg. of gamma globulin of the "negative" pool. The reagent still contains antibodies to the major human gamma globulin antigen. (The nonspecific band which forms laterally to the starch gel pieces can be discounted in assessing the specific precipitin reactions.)

[1]These types do not appear to be the same as Grubb's Gm groups, as indicated by the reactions of 12 human sera (generously given to me by Dr. R. Grubb and split equally between his two Gm groups).

versity of Michigan.[2] So far, five identical twin pairs and six fraternal pairs have been analyzed. There is a photographic reproduction in figure 2 of the agar precipitin reactions afforded by one of the twin pairs and their parents, simultaneous comparison being effected in one chamber. Concordant results have been obtained with each pair of identical twins, two pairs yielding clear cut positive reactions and three pairs yielding negative or weak reactions. On the other hand, seven of the parent sets and two of the fraternal twin pairs have yielded discordant reactions. The gradation of reactions in many samples between a clear cut positive and a clear cut negative will no doubt complicate the genetic interpretation, which will be withheld until a larger number of subjects have been analyzed. Nonetheless the data support the contention that genetic information reflects itself in these antigenic configurations occurring (when found in a human serum sample) on only a fraction of the gamma globulin molecules.

It appears, then, that chicken antiserum reagents may provide useful tools in the search for intraspecific differences in the specificities of mammalian proteins. The results suggest that in the epigenesis of human proteins certain antigenic specificities, which continue to evolve long after birth, are shaped in part by late-acting genes. The full implications of this suggestion are outlined in the following theoretical constructions. These, it should be emphasized, are derived from data already in the literature, irrespective of that gathered with the chicken antisera.

THEORETICAL

The initial postulates are:

(1) There is an antithesis between the rapidity of cellular replication (involving the "auto-catalytic" activity of genes) and the degree of transference of inherited information from the genes into the specificities of proteins (involving the "hetero-catalytic" activity of genes).

(2) In response to specific stimuli there is transference from the genes of that portion of the inherited information needed for the synthesis of the appropriate protein types with which the living system maintains its integrity against the disintegrating influences of the outside environment.

These statements lead to the view that much less genetic information is expressed in protein synthesis in early ontogenetic stages than in the later stages and that the full expression of the genetic information in the shapes of proteins occurs only after the mature stage of ontogeny is reached. In this regard, as can be deduced from Brachet (1950, 1957), Warburg (1956), and Crick (1958), the undifferentiated state (for example, of the early embryo) with its anaerobic respiration and its low redox potential and small amount of fixed cytoplasmic structure is associated with rapidly dividing cells; whereas the differentiated state (for example, of the adult) with its

[2] I am indebted to Dr. H. Eldon Sutton for making available a copy of this list with its detailed work-up of the twins, and to Dr. Ernst Rodin for help in obtaining the serum samples from the twins and their parents. Clark (1956) has published information on these twins.

aerobic respiration and its increase in redox potential and in fixed cytoplasmic structure is typically associated with non-dividing cells engaged in prolific synthesis of highly specific proteins.[3]

Thus this view, which takes cognizance of the dynamics of cellular replication, offers an explanation for recent experiments (Conway et al., 1958; Toolan, 1958; Snyderman, 1958) concerning the transplantation of embryonic and fetal tissue into mammalian adults. These experiments, in which successful transplants of human embryonic skin into adults occurred (Toolan, 1958, and Snyderman, 1958), suggest that the histocompatibility antigens are less well formed in the embryo than in the adult. Loeb (1945) in reviewing earlier data pointing to the same conclusion derived his concept of "organismal differentials" as protein substances which become structurally more complex as the forms of life in which they occur increase in phylogenetic and ontogenetic status. It can be readily seen that if adult proteins contain more genetic information than embryonic proteins, they would exhibit more fully the finer "organismal differential" properties underlying biological individuality.

A factor, decisively affecting the evolution of individuality, is the antigenicity of proteins. The tissue system which recognizes antigenicity appears to be restricted to the later developmental stages of the vertebrates, for the responsible nonphagocytic lymphocytes and plasma cells are not present in the invertebrates nor in the early embryos of vertebrates (Favour, 1958). These cells are generally thought (Burnet, 1956) to arise from a more primitive mesenchymal (macrophage) system in the developing vertebrate as an evolutionary adaptation to permit acquired immune responses to the antigens of outside invaders. Once this mesenchymal system differentiates into one capable of yielding immune responses, it will tend to recognize upon challenge the antigenicity of any protein specificity with which it has not been in prior systemic contact. Thus the danger arises that a mother may respond immunologically to the child in utero and the post-natal individual may respond immunologically to autologous proteins. The danger is certainly acute in the primates with their long gestation period and their finely developed "organismal differentials." Its consequences, so far as certain facets of human evolution are concerned, can be summarized in a two part hypothesis, as follows:

(1) Although a tendency towards genic heterogeneity exists among human beings, apparently imparting survival value in a changing environment, a basal level of genic homogeneity in the population is insured by immune reactions on the part of the mother to the child. (Thus, by virtue of this immunological mechanism, the law of parallel embryological development as

[3]Brachet, of course, deals extensively with the intracellular chemical changes that characterize the mitotic cycle and the progression of embryogenesis. Warburg, in particular, emphasizes the relationship between the mode of respiration, the amount of fixed cytoplasmic structure, and the state of differentiation of the cells concerned. Crick, indirectly, contributes to the generalization presented by referring to observations which associate the prolific synthesis of secretory proteins with cytoplasmic structures (such as microsomes and the endoplasmic reticulum).

seen to operate in mammalian species can readily be derived from the mutation-natural selection theory of evolution.) The smallest number of mutant or allelic forms will exist in the human population for the genes which (concerned with the endogenous adaptations of the organism) become active in protein synthesis during the early stages of ontogeny. The largest number of mutant forms will exist for those genes which (concerned with exogenous adaptations) become most active in protein synthesis during the later stages of development. Furthermore, there will be a selective advantage for those genic mutants which by expressing themselves strongly in protein synthesis only after the child is born escape the mother-fetus immune relationship.

(2) The genes concerned with gamma globulin (antibody) synthesis fall under this category. Too early an ontogenetic expression of these gamma globulin genes would cause the individual to respond immunologically to maternal antigens and also to his own developing antigens. Too late an ontogenetic expression of these genes would endanger the individual's survival chances in the face of hostile invaders from the exogenous environment. Natural selection has only partially resolved these two antagonistic pressures, of which the former is intensified by a delayed epigenesis of certain protein antigens due to the maternal immunological pressure. The resolution is such that man has an imperfect tolerance to his own proteins, and potentially can be stimulated by a further epigenesis of these proteins to self destructive reactions. Thus the further evolution of biological individuality (finer "organismal differentials") depends upon the ability of man to effect a new resolution, one that prolongs the neonatal period for acquiring a primary state of immunological tolerance to autologous proteins.

Several types of experimental results can be cited to support the first part of the hypothesis. Brambell (1951, 1958) has summarized data which demonstrate that embryonic and fetal proteins gain access to the antibody producing cells of the mother and that maternal proteins (gamma globulin in especially large amounts) cross over via the placenta (Bangham et al., 1958) into the primate fetus. In this regard, it has been shown (Thomas et al., 1959) that human fetal trophoblasts routinely enter the maternal blood stream. Relating this kind of data to the clearly established cases of an immunization of the mother by the fetus in the Rh and ABO incompatibilities, Brambell et al. (1951) and Nace (1957) (and no doubt others) have concluded that embryonic injuries and deaths due to isoimmunizations by protein antigens as distinct from the blood group antigens must also be of common occurrence. The genes responsible for shaping the potential isoantigens of the fetus are, of course, those of the paternal haploid contribution which differ from any in the genotype of the mother and which express themselves in protein synthesis early in ontogenetic development.

The first part of the hypothesis, then, focuses attention on the type of protein antigens most apt to demonstrate the process of evolutionary change among human beings. It may have heuristic value in that it provides a framework for attempting to relate the data of immunoembryology to that of systematic serology. For example, immunoembryological investigations (Nace,

1953) have shown that serum albumin arises early in vertebrate ontogeny whereas gamma globulin arises late. According to the hypothesis we would predict that within a primate species and perhaps even between closely related primate species serum albumin as an antigen would be characterized by its homogeneity in the population and gamma globulin by its heterogeneity. In this regard, Boyden (1958) has reported systematic serological investigations which show that there is a higher degree of correspondence for the serum albumins of closely related mammalian species than for the serum globulins. Goodman (1959b) cites recently obtained data which directly verify the prediction. It is of interest that Dray and Young (1958) with isoprecipitins produced against whole rabbit serum could detect serum isoantigens of gamma, beta, and alpha mobility but not any of albumin mobility. They (1959) and Dubiski et al. (1959) have shown (as already mentioned) that gamma globulin isoantigens exhibit genetic variation.

One of the more interesting observations which can be cited in favor of the hypothesis has been brought to light by the studies (for example, those of Simonsen, 1957; Billingham, 1958; Trentin, 1958) concerned with the homografting of adult spleen or bone marrow cells into neonatal recipients or into severely x-radiated recipients. A syndrome which developed in many instances has been shown to result from an immunological attack on the host by survivors of the homografted cells. In the case of the neonatal recipients, this syndrome is called runt's disease, and the major site of the pathology is the lymphoid tissue of the host. Even in homografted animals without gross evidence of runt's disease, an involution of autologous lymphoid tissue has been found. Here, then, we have a dramatic example of an isoimmunization in which the richest source of antigens appears to be that part of the host's mesenchymal tissue arising last in embryogenesis.

An ontogenetic delay in the appearance of such antigens until after birth would serve to resolve the antagonism caused by the potential for the mother to engage in immunological aggression against her child and the impelling necessity for genic heterozygosity if human beings are to exist in a broad range of a changing exogenous environment. As pointed out in the hypothesis, the delay could be effected by a selection for mutant genes which would wait until after birth before partaking actively in protein synthesis. As a consequence of such genes, however, man would suffer from an intensification of the self-destructive tendencies unleashed by the rapid, neonatal maturation of his immunological system.

The picture sketched in the second part of the hypothesis is one in which these self-destructive tendencies congeal shortly after birth and then so affect the individual in his subsequent growth as to hasten his ultimate decay. Emphasis is placed on the incompleteness of the full antigenic development of the "organismal differentials" in the neonatal individual at the time that autologous antibody synthesis begins. Thus, issue would be taken with Voisin et al. (1958) who, in presenting the typical view of the organ specific nature of autoantigens, argue that "the individual specificity that results in homograft antigenicity seems to be precisely the factor that prevents

an antigen from being autoantigenic." The traditional belief which they express that the genetic constitution "will not normally build proteins foreign to the organism" is probably still widely held. Yet, this belief falls down if one attempts to relate known examples of auto-immunization (Waksman, 1959) to the experimental demonstration that immunological tolerance of a sort can be acquired against genetically foreign substances (Owen, 1957). It would be more explanatory to consider (from the definitions of Burnet, 1956; Medawar, 1957) the primary state of immunological tolerance as one restricted solely to those structural groupings of proteins and like substances which had been in systemic contact with the progenitors of the immunological system at some time prior to the initial activation of the antibody synthetic mechanisms.

Once accepting this view, we are forced to conclude from the postulates upon which the above hypothesis is based that as more and more genes become active in protein synthesis under the impact of changing environmental stimuli (endogenous as well as exogenous with the growth of the individual), there is a mounting synthesis of autologous structural groupings not recognized as native by the immunological system. Thus, it is precisely in the nature of our genetic constitution as evolved to normally build proteins, even circulating ones, which behave as foreign substances in the body. The situation depicted is not quite so catastrophic as it seems, for (to follow the logic employed by Owen et al., 1954) immunological tolerance is probably engendered in the fetus to the predominant specificities of the circulating proteins of the human species, even those which arise late in ontogeny, by the passage of maternal proteins across the placenta. Thus, again, a key factor in the causation of variant specificities (which in this case are autoantigenic rather than isoantigenic) are those genes in the paternal haploid contribution which differ from any in the genotype of the mother. But this time we are concerned with genes which, in imparting survival value to the organism faced with a hostile outside environment, express themselves in protein synthesis late in the developmental phases of ontogeny rather than early.

A schematic way to analyze the potential severity of the auto-intolerant state is to first devise in theory an epigenetic protein taxonomy along the lines of animal taxonomy. The proteins of an individual would be grouped together into epi (for epigenetic) phyla, classes, orders, families, genera, and species on the basis of structural affinities (which in turn might be related to degrees of overlap in the portions of genetic information shaping the various protein specificities). Then the severity of the initial auto-intolerant reactions at the time that antibody synthesis begins would be determined by the extent of the taxonomic disparity between newly arising protein specificities and those to which immunological tolerance had previously been engendered. For example, if several or more of the systemically available proteins showed this disparity at the epi-family level, severe reactions could be triggered whereas at the epi-subspecies level, in all likelihood, autosensitization would be negligible.

In the first cited example, the infant (a victim of environmental accident or genetic endowment) could presumably perish under the impact of runaway self-destructive antibody reactions. Or, as seems more probable, compensatory physiological mechanisms would come into play to repress such antibody reactions and permit development of a secondary state of tolerance to the inciting proteins. Nonetheless, the stressed individual on consuming some of his store of "adaptation energy" (to invoke a concept from Selye, 1952) would be conditioned to respond in a different manner to protein challenges than the individual who acquired a more adequate primary state of tolerance to autologous proteins. We have but to consider the superstructural complexity in the higher vertebrates of the hormonal and neuromimetic agents which affect the functioning of the immunological system to see that these responses would be idiosyncratic in a pronounced way in the first type of individual. Thus, as ontogeny progresses, the new protein specificities which constantly arise must contribute as potential immunological irritants to the ultimate onset of full-blown degenerative disease, overcoming the first type of individual sooner than the second. Again, the unique features of the pathogenic consequences of individuality would in each individual be determined both by environmental accident and genetic endowment.

These suggestions of how self-destructive tendencies take hold early in life point to the inadequacy of current efforts to cope with human suffering. In essence, from the view presented, we face the challenge of changing our conditions of life if we are to make significant progress in combating degenerative disease in all of its organic and mental manifestations. For one thing, we must exert such mastery over our environment on the earth as to drastically reduce the assaults on our immunological system (such as from the perverted protein synthesis induced by chemical carcinogens, or x-radiation, or viral nucleic acids) and perhaps, by this means, cause a much slower maturation of the antibody synthetic mechanisms of the newborn. Our thesis, then, leads to the conclusion that to evolve biologically in a favorable direction we must begin with our present genetic heritage to create the situation which allows for a more deeply rooted tolerance in our inmost beings.

SUMMARY

Antigenic components as subfractions of serum gamma$_2$ globulin were demonstrated by the immunoelectrophoretic reactions of certain chicken antisera. These components were found in some human beings but not in others, and their analogous nature to the "organismal differentials" of Loeb was pointed out. A two part hypothesis concerning the effects of protein antigenicity on human evolution was presented. The first part showed how the maternal immune system could act as a selective agency against mutant genes expressing themselves early in ontogeny and thus account for the law of parallel embryological development. The second part of the hypothesis showed how a delayed epigenesis of protein types, due to selection in favor of mutant genes expressing themselves postnatally, would cause man to have an imperfect immunological tolerance to his own proteins. That such in-

tolerance would be a potent factor underlying degenerative disease was discussed.

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MICROBIOLOGY, DEVELOPMENTAL GENETICS AND EVOLUTION*

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I do not intend to trace, even in brief outline, all the complex interactions of developmental and evolutionary processes as they are interlocked in genetic mechanisms. Instead, I plan to comment on one critical stage in the evolutionary sequence, the transition from the unicellular to the multicellular mode of existence. More specifically, I wish to inquire about which of the elementary developmental processes originated prior to the establishment of the multicellular habit, and which afterward, what the functions of such processes might originally have been, and how the mechanisms might have operated.

Such an inquiry cannot, of course, be instituted directly, for both the ancestral unicellular forms and the ancestral multicellular forms departed long ago, leaving little morphological record and essentially no physiological trace. As in many other evolutionary investigations, one can only study extant forms and hope that their properties can provide some insight into the ancestral conditions.

Such an investigation becomes, then, a comparison of the developmental mechanisms in living unicellular and multicellular organisms. It assumes, of course, that comparable mechanisms exist in the forms to be compared, and the first task is to establish the existence of developmental mechanisms in micro-organisms. This task is not so difficult as one might imagine, for in recent years microbiologists working with a great variety of organisms have discovered phenomena which appear in all major respects similar to those in higher forms.

DEVELOPMENT

To simplify the comparisons, development in a higher organism can be described in terms of a small number of elementary processes (figure 1). First, beginning with a fertilized egg, a population of cells is produced. The details of the mitotic cycle assure, with certain conspicuous exceptions, that the resulting cells are alike in their genetic endowment. In spite of their genetic identity, however, the cells from a single source become different from each other — in morphology, in physiology, in specific biochemistry. That the cells should become different is not

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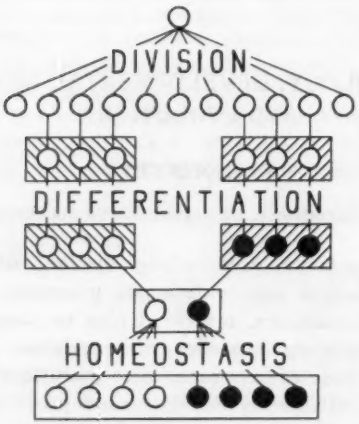

FIGURE 1

surprising, since they occupy different micro-environments in the embryo; and phenotype — even at the cellular level — has long been recognized to be a function of both the genotype and the environment. The more surprising fact is that many of the cellular differences, once established, are remarkably resistant to obliteration. After a certain stage of development, characteristic for the tissue and the organism, cells from different parts of the embryo transplanted to a common site or explanted into tissue culture may not become alike, but maintain their individuality. They "remember" the former conditions of their existence and refuse to alter the course of development upon which they have embarked. Hence, cells alike in genetic constitution, living in the same environment, may remain different from each other for many cell generations. This persistence of cellular differences in the absence of genetic or environmental differentials may be called "epigenetic homeostasis" (Nanney, 1958a; Ephrussi, 1958); the underlying mechanisms constitute a major problem for analysis.

Turning to the unicellular forms, one observes a series of striking similarities. One may start with a single cell, a bacterium or a protozoan, and derive by processes analogous to those in higher forms a population of genetically identical cells. The chief distinction between an embryo and a clone of microorganisms resides in the fact that the cells are dissociated in the latter case; and this distinction may not be of fundamental significance. One may now subdivide the population into groups of identical cells and expose them to different environments, even as embryonic cells inhabit different local environments and as populations of microorganisms are distributed in nature over a variety of habitats. Again, as in the embryo, the cells derived from the same source become different in the different environments, even though they maintain the same genetic constitutions. Finally, one may return the differentiated cells to a common environment and permit them to multiply. In many instances the differences

established by exposure to different environmental stimuli are maintained thereafter indefinitely in cellular multiplication. It is important to note that the genetic identity assumed for embryonic cells can often be directly established for differentiated microbial cells by breeding analysis. The conclusion that phenotypic diversity may persist in the face of genetic identity is, therefore, on even firmer grounds in microorganisms than in higher forms. Epigenetic homeostasis is thus not a phenomenon restricted to higher animals, but occurs fully developed in unicellular organisms. Indeed, the evidence for cellular heredity in microorganisms is, thus far, even more compelling than that in higher forms.

EPIGENETIC HOMEOSTASIS

Before going further with this argument, I would like to depart from generalizations long enough to provide two illustrations of microbial epigenetic homeostasis, one established several years ago and another more recently. The first derives from studies of Sonneborn, Beale and their co-workers with *Paramecium aurelia* (See Beale, 1954, 1957). A single paramecium (Syngen 1, stock 60) is allowed to multiply (figure 2). After a clone is established it is split into two portions, one placed at 15°C, and the other at 25°C. After a short period all the cells in the first population manifest an antigen designated as S, while those in the second population manifest a different antigen, G. When the cells from the two populations are now placed in identical environments, specifically at 20°C., the cells do not become alike, but maintain their differences indefinitely. The cellular states, established by exposure to different environmental stimuli, become self-maintaining in the absence of the environmental differentials.

The second example is drawn from the extensive studies on induced enzyme sythesis in the bacterium, *Escherichia coli* (See Cohn, 1956;

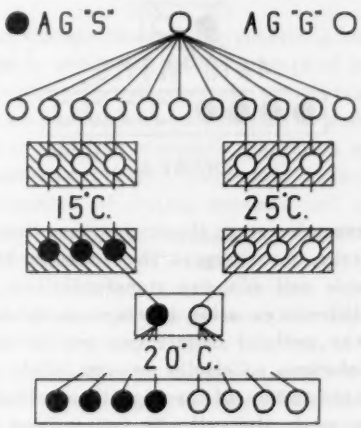

FIGURE 2

environment and permit them to multiply. In many instances the differences

Novick and Weiner, 1957). It is concerned with the production of an enzyme, designated as a "permease", responsible for bringing sugars of a particular kind into the cell (figure 3). A clone with the genetic capacity to produce this enzyme may be divided into two populations, one of which is exposed to a high concentration of a specific inducer (4×10^{-4}M thiomethyl-Beta-D-galactoside) while the other is maintained in the absence of the inducer. After a few divisions all the cells in the first population are actively producing the permease, while none of those in the second population is doing so. If cells from the two populations are now placed in the same environment, specifically one containing a very low concentration of the inducer (4×10^{-6}M TMG), the cells from the first population continue to produce the enzyme and those from the second population fail to do so. The differences between the two kinds of cells have been maintained in identical environments for at least 180 cell divisions. Again the persistence of the cellular differences can be attributed neither to differences in the genes nor to differences in the external environment, and a mechanism of epigenetic homeostasis must must be invoked.

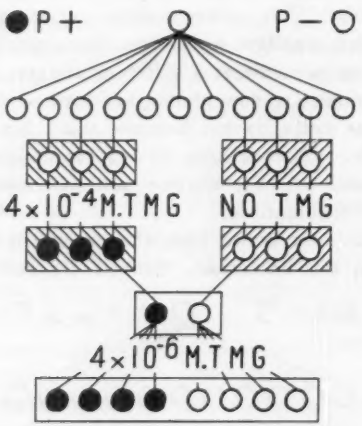

FIGURE 3

These particular examples were chosen because they not only exemplify epigenetic homeostasis, but suggest the intimate relationship between epigenetic homeostasis and adaptive transformations. In each of these cases the cellular differences arise in response to definable conditions, and in each case the cellular differences can be cancelled by further environmental manipulations. Cellular memory is not an absolute quality, but is dependent on the nature of the signals received by the cell. If the extrinsic signals are weak, the cell may ignore them and respond only to the signals of an intrinsic feed-back system; if the extrinsic signals are

FIGURE 2

stronger the cell may transform into another state. In other cases, as with most embryonic inductions, the effective environmental stimuli are not known, and cellular differences cannot be obliterated by known procedures. In still other cases common conditions for stability of different cell types have not been established, and the systems involved appear not to show homeostasis. These apparent differences in cellular responses may reflect only present ignorance and cannot be considered critical.

Incidentally, the suggestion (Grobstein, 1959) that induced enzyme formation may be fundamentally different from embryonic induction in that low molecular weight compounds are involved in one case, high molecular weight compounds in the other, need not be taken seriously. In neither case is the molecular weight of *effective* units known with certainty. Recent studies on enzyme and antibody induction with identical haptens (Tannenbaum *et al.*, 1959) and with primary and secondary antibody responses using closely related haptens (Dubert, 1956) suggest an essential similarity in at least these two "developmental" processes.

DEVELOPMENTAL PROCESSES IN MICROORGANISMS

Certainly many of the components of development in higher forms exist in unicellular organisms — specifically, cell division by a process analogous to mitosis; cellular differentiation in response to extrinsic (or occasionally intrinsic) stimuli; and finally epigenetic homeostasis.

Since such mechanisms exist in modern microorganisms, it is plausible to assume that the ancestral forms from which the metazoa are derived had also developed such mechanisms. If so, the transition from the unicellular to the multicellular form posed problems in complexing existing mechanisms and adapting them to novel ends, rather than in elaborating a large variety of new processes. What were the original functions of cellular differentiation, and what ends does it serve in modern microorganisms?

The elaboration of storage systems for genetic information had certainly occurred much earlier in evolution, for the storage of information in nucleic acids and the replication of nucleoprotein complexes were essential for heredity, without which evolutionary progression is unthinkable (See Muller, 1947). Probably also multiple metabolic pathways had been elaborated, perhaps by the Horowitz (1945) "back-step" scheme for biochemical evolution. The existence of coding systems and multiple biochemical capacities both posed problems and exposed opportunities. Originally, perhaps, all the information in the genetic library required translation at every cell cycle, and all the proteins and other substances formed were essential for the survival of the organism. An organism which merely produces all the required enzymes is not, however, a very efficient organism; some regulation of the amounts of various enzymes must have occurred, perhaps in part by selection of genes with optimal rates of activity under some environmental conditions. But environments are not constant, and as cellular organization became more elaborate, and competition became

more rigorous, more and more "dispensible" functions were acquired; substances were formed which were not needed all the time or under all circumstances, or which were required in different amounts at different times.

At this point the primitive cells faced serious economic problems. While a particular enzyme would confer on the cell producing it a distinct advantage when its substrate was present, in the absence of the substrate the enzyme would be excess baggage; it would require raw materials and energy for its elaboration, but it would serve no immediate end. Hence, an organism with enhanced enzymatic capacities would under some circumstances be at a disadvantage in competition with its "stripped down" cousins. The solution to the problem was to store the blue-prints for the enzyme in the genetic library, but to produce the enzyme only under conditions in which it could be used. The expense of storing information in a single nucleoprotein molecule is slight in comparison with the cost of maintaining thousands of enzyme molecules and all the means of their production. The chief new expense was that of installing a system of switches which would terminate production of a particular substance when it was not required, and which would initiate production when conditions warranted. The acquisition of flexibility, the capacity to modify metabolic patterns in the face of changing environments, was of advantage to the primitive unicellular forms—even as it is for modern microorganisms—and the system of switches was to be the foundation of the multicellular economy.

Such considerations do not resolve all the "why" problems; they aid in understanding the evolution of metabolic flexibility, but they do not explain the origin of epigenetic homeostasis, the stability of alternative cellular patterns. Why should two cells of the same genotype in the same environment maintain their differences, assuming that the differences are in some sense "adaptive"? Perhaps the significance of microbial epigenetic homeostasis can be found in the dynamics of microbial populations. Unicellular forms are usually considered physiologically independent and their ecological interactions are minimized. Yet, even these organisms participate in a limited social life, and the structure of the population is occasionally important to the individual cell. For example, few if any of these organisms can completely abjure sex, the most intimate of social interactions. Since compatibility barriers usually prevent the mating of identical cells, some regulation of population structure is required if efficient mating is to be achieved. Compatibility differences can of course be established by genetic heterogeneity, but epigenetic heterogeneity can achieve the same ends, in some cases perhaps more effectively. Certainly epigenetic regulation is the foundation of many of the elaborate mating systems in the ciliated protozoa (See Sonneborn, 1957; Nanney, 1958b). The social interactions involved in reproductive activity are shown perhaps even more dramatically in forms like the ameboid slime

molds studied by Sussman (1958). Here the independent cells actually aggregate at a signal from a differentiated cell to form a fruiting structure.

Similar considerations may hold for other physiological interactions in microbial populations. Certainly "cross-feeding" by genetically different organisms has been demonstrated in many cases. One kind of cell may utilize a particular substrate and supply products to another, which may in turn supply still other products to the population. While cross-feeding by genetically identical, but epigenetically distinct cells has not yet been demonstrated in microorganisms, intensive search for such a division of labor has not been made. Certainly the advantages of such an arrangement could be considerable.

One must also consider the response of a population to changing environmental conditions. If environmental changes are gradual, opportunity might exist for switch mechanisms to be activated and for individual cells to modify their physiological states. Under such circumstances no advantage would accrue to the cell which persists in its former state. If, on the other hand, environmental changes are sudden and traumatic, the survival of a population might depend on its heterogeneity. One usually thinks of population adaptation in terms of selection for pre-existing genetic mutants. But mutations must be rare if the genetic system is to perform its stabilizing function; if a population is small, the proper mutant might not be present at all. Epigenetic variation, adjusted to occur at a higher rate than mutation, would be helpful in enabling populations to adjust, particularly to commonly encountered environmental transitions. Some evidence is indeed available for such uses of epigenetic variation in microorganisms.

This problem is not so acute in haploid populations where mutations are immediately expressed, but becomes a serious problem in diploid populations, where both homologous loci might have to mutate in the same way in order to yield a pre-adapted cell. One might expect that diploid cells would rely more generally on epigenetic devices than haploid cells. It is perhaps significant that the step from unicellularity to multicellularity has generally occurred in diploid cells, i.e., those cells in which epigenetic systems might be more fully developed.

ORIGINS OF EPIGENETIC HOMEOSTASIS

Finally, we may ask how microorganisms achieve cellular differentiation and epigenetic homeostasis. This is an important question, not only for an understanding of microbial variation but also for an understanding of cellular differentiation in higher forms. Unicellular organisms offer some distinct advantages in experimental analysis. In the first place the cellular environment can be controlled with great precision, while the significant environmental conditions surrounding embryonic cells can usually not even be defined, much less controlled. The serotype studies on *P. aurelia,* and the permease studies on *E. coli,* mentioned earlier,

provide fruitful examples of environmental manipulation. Even more significantly, however, differentiated microbial cells can often be crossed and the bases for cellular differences can be explored with the powerful tools of genetic analysis. The great variety of sexual and parasexual mechanisms in microorganisms provides a battery of experimental techniques which cannot be matched in the relatively standardized cytogenetic apparatus of higher forms.

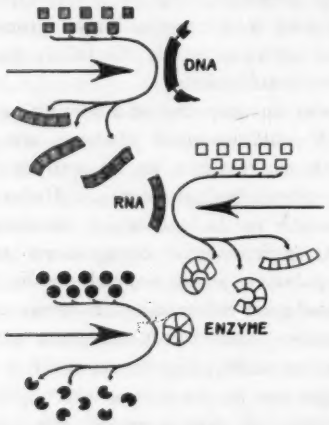

FIGURE 4

Unfortunately, the studies now available on microbial variation do not provide a clear simple basis for all epigenetic alterations. The apparent differences may not be real and a common denominator may yet emerge. At the present time one could develop any of several unifying concepts by judiciously selecting case histories. It is my intention to outline one such hypothesis — that epigenetic alterations are primarily due to alterations in the nuclei, and more specifically of the nuclear constituents associated with the chromosomes. The first evidence in favor of such an hypothesis is theoretical, and is an argument based on the cellular economy. According to the currently popular working hypothesis concerning cellular processes, the information for specific synthesis lies initially in the nucleoprotein complexes in the nucleus, perhaps coded in nucleotide sequences in the DNA (figure 4). This information is translated into secondary templates of RNA, and often packaged in the form of ribosomes to be delivered to the cytoplasm. Finally, the information is again translated into amino acid sequences in polypeptide chains and ultimately into enzyme specificities. Interference with a particular synthetic sequence could occur at any of these levels. The DNA could be prevented from transferring information to RNA, the RNA could be blocked from its task of ordering amino acids, or enzymes could be in-

activated by combining them with analogues of their normal substrates, to mention a few of the possibilities. We have already suggested that terminating particular syntheses can be construed as an economy measure; if so, one might expect the switch mechanisms to block the syntheses in the most economical way possible. To inactivate enzymes is not economical; a cell may contain thousands of enzymes of a particular kind; both forming the enzymes and forming the inactivators are costly processes. Similar considerations hold for the ribosomes; a cell may contain hundreds of replicates of each kind of RNA, and no advantage derives from manufacturing them and then inactivating them. Hence, the logical candidates for suppression are the DNA complexes, the genes themselves. A cell will usually contain only one or two examples of a particular gene; locking up the blueprints appears to be the cheapest way of regulating particular processes.

Such arguments are of course only suggestive and would have to be discounted if experimental evidence were not available to support them. But such evidence is available. The evidence for nuclear differentiation in development in higher forms is, of course, well known (Briggs and King, 1959). One of the more significant of recent studies on microorganisms is that of Lederberg and Iino (1956) on serotype variation in salmonella. Many strains of this organism are "bi-phasic", that is, any clone, orginating in a single cell, eventually contains two kinds of cells distinguished by the antigens borne on their flagella. Genetic analysis demonstrates that the specificities for the two antigenic types are controlled by two distinct loci, the H_1 and the H_2 loci, only one of which is expressed at any one time. The conditions required for switching a cell from the active H_1 state to the active H_2 state, and *vice versa*, have not yet been worked out. The changes do occur "spontaneously", however, at a rate characteristic for a particular strain. Usually the changes occur much more frequently than would be expected for conventional mutations. That genetic mutations are not involved is also suggested by the fact that the changes are reversible and all the changes are between the two original specificities.

SEROTYPE TRANSFORMATIONS

For these and other reasons it appears that the serotype transformations are epigenetic, and that an understanding of these cellular differences may provide a clue to cellular differentiation in other forms. The analytical procedure employed in this case was a special type of "nuclear transplanation"; small fragments of genetic material were transferred from a differentiated cell of one genotype to a cell of another genotype, by means of a virus vector. Since transducing viruses can be prepared from cells in either of the differentiated states, and exposed to common recipient cells, the relative roles of the stripped, or nearly stripped, "chromosomal" material and of "extrachromosomal" materials in the

control of the serotypes can be evaluated. If the differences between cells were entirely extra-chromosomal, the source of the genetic materials should not be significant in determining the character of the progeny. If, on the other hand, the serotype differentiation involved changes at the level of the chromosomes themselves, one might expect different results depending on the source of the fragment. This was indeed found, and the inference is unmistakable; the cellular differences, though expressed as differences in flagellar antigens, are under the control of switch mechanisms located within or upon the chromosomes.

All such studies do not, of course, yield such unequivocal results. In particular, many examples of cellular heredity in microorganisms appear to have cytoplasmic rather than nuclear bases (Sonneborn, 1950; Ephrussi, 1953). Some recent studies suggest, however, that this apparent discrepancy need not be fatal to a unitary hypothesis. Particularly revealing is Sonneborn's (1954) analysis of mating type determination in *Paramecium aurelia*. Two groups of genetic species or "syngens" occur within the taxonomic species. In one group mating type determination clearly involves a nuclear differentiation; in the other group the mating types appear on preliminary analysis to be under the control of the cytoplasm. Further study shows, however, that nuclear differentiation occurs in both instances and indeed is the central feature of both. The apparent differences arise from the fact that, in one case, the cytoplasm can carry messages from one nucleus to another, instructing an uncommitted nucleus to a particular developmental pathway. Once differentiated, however, the instructed nucleus maintains its character and the passive cytoplasmic "messengers" are rapidly diluted out.

One last example must suffice. Nearly all the studies on serotype determination in Paramecium indicate that the cytoplasm plays an important role in the control of cellular differences, even though nuclear genes are well established as the source of antigenic specificities (See Beale, 1957). In the closely related ciliate, *Tetrahymena pyriformis*, serotype differentiation also occurs, and in most of its aspects appears strictly comparable to that in Paramecium (Margolin *et al.*, 1959; Inoki and Matsushiro, 1958). However, certain of the serotype differences, specifically differences between heterozygous cells expressing different alleles of the same locus, are perpetuated by stable nuclear alterations, and no trace of cytoplasmic influence has been detected (Nanney, 1959).

Thus, the study of similar cellular traits in closely related organisms may yield on initial analysis a picture of great diversity of mechanisms. In some cases the cytoplasm appears to control cellular differences and in other cases the nucleus seems to play such a role. On more careful analysis, however, the differences are often found to be superficial and nuclear differentiation emerges as the common feature in apparently diverse systems.

Other examples of apparent "cytoplasmic heredity" may have similar explanations. Even for such obvious cytoplasmic organelles as plastids,

the evidence for a major role of the cytoplasm in perpetuating differences is not compelling (von Wettstein, 1959). Most analytical procedures do not distinguish between the cytoplasm as a passive route of information transfer, and the cytoplasm as an active participant in homeostasis.

The available studies thus demonstrate that some cellular differentiation in both microbes and higher forms involves nuclear differentiation, and suggest that such differentiation is a primary factor in the regulation of genetic expression. But a geographic localization of control systems is only a first feeble step toward an understanding of mechanisms. What is the difference between an active genetic locus and an inactive locus? How is its activity status maintained? What is the nature of the "messenger" which instructs a locus on its level of activity? Where is the messenger produced? On such questions one can only speculate. It has been suggested (Markert, 1958) that a segment of DNA is activated when it combines with a specific protein. One might argue alternatively that a gene is inactivated when it combines with a specific protein, or perhaps more likely with a particular species of RNA. This formulation has some attractive features. One of the major characteristics of differentiation in both higher and lower forms is mutual exclusion; the formation of one kind of protein (and RNA) often precludes the formation of another. If the RNA or protein specified by one genetic locus could combine with and suppress the activity of another locus, many problems would be solved simultaneously: the origin of a messenger, the integration of genetic activities, the establishment of a "feed-back" system.

Perhaps speculation at this level is improper at the present time, but the prediction that a further understanding of developmental mechanisms can come through a broad comparative study of many kinds of organisms seems fully warranted.

SUMMARY

The major elementary components of development in higher forms, that is, cellular division, cellular differentiation and epigenetic homeostasis, are well established in microorganisms. Their existence and functional significance in modern microbes suggest that they may all have arisen in primitive unicellular forms and may have provided the "pre-adaptive" basis for the transition to multicellularity. Regardless of whether the developmental mechanisms in unicellular and multicellular organisms are parts of a single evolutionary sequence or represent parallel evolutionary advances, modern experimental analyses emphasize their essential similarities. Nuclear differentiation, the regulation of genetic activities by modifications at particular chromosomal sites, may prove to be an important unifying concept. In any case, broad comparative studies on a variety of forms, selected for their suitability in exploring particular problems, should cross-illuminate each other and provide eventually the foundation for a "synthetic" developmental biology.

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LETTERS TO THE EDITORS

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THE GENOTYPE AS THE SUM OF PLASMATYPE AND CHROMOTYPE

In 1910 the American Society of Naturalists held a symposium on the "Genotype Hypothesis," an indication of the importance of the ideas associated with the name of Wilhelm Johannsen. The genotype conception is based on the fact that individuals which appear alike may be germinally unlike and the reverse. This well known situation follows directly from Mendel's laws of inheritance. In his paper presented to this symposium Johannsen was not convinced by the new evidence that genes were located in chromosomes. He considered it relatively unimportant where the hereditary determiners were positioned.

Since it has now been established that there are transmissible differences that are not distributed with the chromosomes (see review by Caspari, 1948, and Michaelis, 1954), an extension of Johannsen's terminology seems to be needed. In some of the lower organisms transmissible units can be seen outside the nucleus, measured and counted (Preer, 1950). In the higher plants no particles, known to carry genetic information, outside the chromosomes or plastids, can be identified. However, differences do exist. For example, in maize three different cytoplasmic conditions affecting pollen production or abortion have been identified and have been shown to bring about chemical differences at certain stages of microspore development (Jones, Khoo and Stinson, and Khoo and Stinson, 1957).

Pollen abortion results after meiosis when certain genic combinations are placed with different cytoplasms by crossing and backcrossing. Genes in the chromosomes are known to restore some, but not other, combinations of plasmatype and chromotype to normal fertility. Some of the genes for normal pollen production are different for each type of cytoplasm so far identified by test crosses (Jones, Stinson, Khoo, 1957).

When Rhoades (1933) first studied pollen abortion in maize he found no clear genic control of this condition. As Sonneborn (1949) pointed out, there was no way of detecting gene control as long as all of the strains investigated had the same genes. As soon as pollen-restoring genes were discovered (Jones, 1950) the interrelation of cytoplasmic and nuclear determiners became apparent. Pollen restorers make possible at present the differentiation of at least three different types of cytoplasm in maize.

These differences, while under genic control, are also conditioned by something outside the chromosomes. Whatever they are that bring about these differences they are transmissible, autonomous and stable in different chromotypes for all of the many generations they have been followed (15 in maize). Since these hereditary factors (plasmagenes) have not been located there is no way of knowing, as yet, where or what they are. Since they are

not transmitted by the male germ cells they presumably are not in the nucleus and must be in the cytoplasm or distributed with it. The cytoplasm is usually not transmitted by the male germ cells to the fertilized egg in an amount large enough to be detectable.

In green plants the plastids that control chlorophyll production are known to differ and these differences are transmitted through the egg, presumably by plastid primordia that have continuity from one generation to the next. Like pollen abortion most chlorophyll differences are also under genic control but again the plastid differences are transmissible, autonomous and stable. These differences have been attributed to plastogenes (Iami, 1937) or chondriogenes (Woods and DuBuy, 1945). Pollen abortion seems to be in a different category so the term plasmagene is being more and more frequently used for factors of this type. Cytogene, genoid, plasmid and other terms have been used. Pontecorvo (1946) suggests that all these extranuclear factors be called plasmagenes.

Although the cytoplasmic factors in maize and other organisms are under chromogenic control it is equally true that the chromogenes are also subject to cytoplasmic regulation. In other words, they are both mutually interdependent and both types of controlling factors can remain in combination for an indefinite number of generations without alteration. At least the evidence from higher plants supports this (Jones, Stinson, Khoo, 1957).

It now seems necessary to extend genetic terminology to include both chromosomal and non-chromosomal hereditary material. All of the terms listed in the accompanying table have been used in publications from time to time, some with different meanings. They are brought together here for tentative consideration. Only continued usage will determine their usefulness.

This terminology does not include any provision for nuclear factors that are not chromosomal, factors which may be in the nucleus but are not distributed with the chromosomes. There is evidence that factors of this type may exist. Epigenetic or epinuclear effects may be in this category (Nanney, 1958). The B chromosomes do not follow the usual pattern of A chromosome distribution.

TABLE 1

Units of hereditary transmission

Transmissible hereditary material	Non-chromosomal	Chromosomal	Cellular
Individual autonomous factor	Plasmagene Cytogene Plastogene Chondriogene Genoid Plasmid	Chromogene	Gene
Unit of organization	Plasmon Plastidom Plastom	Genom	Cytom
Sum of all units	Plasmatype	Chromotype	Genotype

Since the chromogenes are responsible for nearly all of the visible differences in living organisms, naturally they have received the most attention in genetic investigations. Within the species the cytoplasm is usually all the same in type and since most species cannot be recombined there is very little opportunity to observe cytoplasmic differences. However, variations in the cytoplasm within taxonomic species are being found to be more frequent than generally believed. Most of these differences bring about very little visible change and are usually difficult to follow from one generation to the next. They are most frequently found by their effect on basic physiological processes involved in such important functions as chlorophyll and pollen production. Although usually lethal to cells or tissues in their aberrant form some can be transmitted by vegetative propagation or by maternal germ cells. In this way they can be followed from one generation to the next.

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THE SPECIES SPECIFICITY OF PROTEINS AS OBSERVED IN THE WILSON COMPARATIVE ANALYSES PLATES

A plan of study, conceived with Wilson,[1] concerned a comparison of the species specificity of mammalian proteins using both rabbit and chicken antisera. The object was to determine which proteins showed the most antigenic variation between related species and which showed the least. In determining this for human proteins it was felt that comparisons within a panel of primate sera would yield the most information.

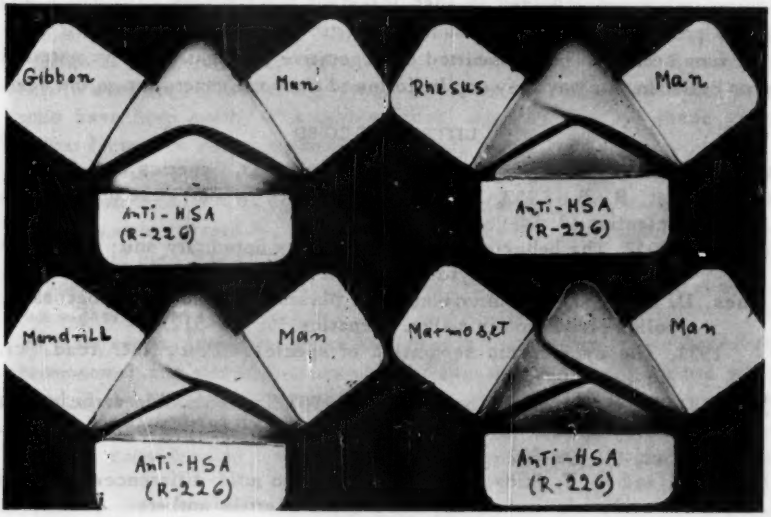

FIGURE 1. Comparisons of primate sera to human serum effected with a rabbit antiserum (R-226) to human serum albumin (HSA).

Wilson first employed a rather typical five basin ouchterlony plate (Wilson and Pringle, 1956). However, he became dissatisfied with the low sensitivity of this type of plate and designed several exceptionally sensitive plates in which the juxtapositioning of the antigen and antiserum basins obviated the dilution effects of the typical plate, thus permitting the weaker precipitin reactions to be demonstrated. Using these new plates, he completed a comparison of the albumins of twelve primate species with a highly specific rabbit antiserum to human serum albumin. The one higher anthropoid next to man in the primate collection was a gibbon, and its serum yielded a reaction of identity with the albumin of human serum, whereas the other primate sera only afforded reactions of partial identity with the human serum. The photographs of some of these reactions are reproduced in figure 1.

Recently I have been able to initiate my part of the originally planned experiments by comparing with chicken antisera, in the most sensitive of the

[1]Dr. Morris Wilson, deceased 1959.

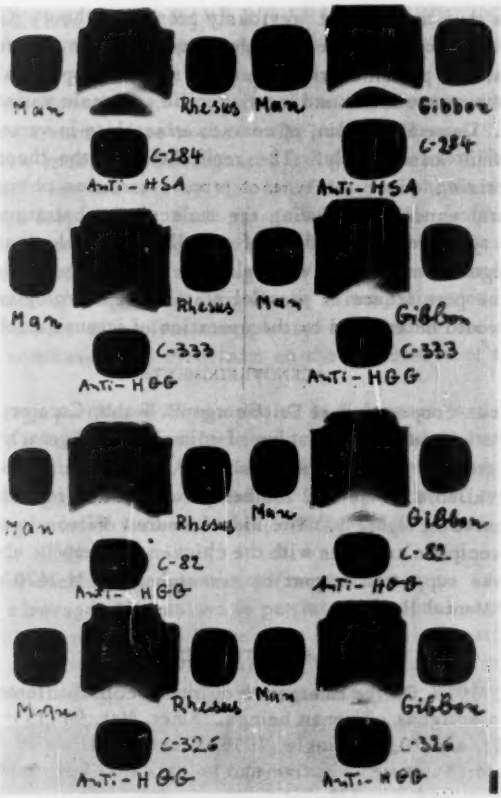

FIGURE 2. Comparisons of primate sera to human serum effected with chicken antisera to human serum albumin (HSA) and to human gamma globulin (HCG). C-284 is a pooled antiserum from five chickens injected with HSA. C-333, C-82, and C-326 are each from individual chickens injected with HGG.

new types of plates designed by Wilson, the serum albumin and the serum gamma globulin of the gibbon and the rhesus monkey to that of man. Figure 2 demonstrates that serum gamma globulin as an antigen exhibits a greater species specificity than serum albumin. Again, as was observed with the rabbit antiserum, a reaction of identity occurred between the serum albumin of the man and the gibbon. On the other hand, with the anti-human gamma globulin sera, some of which were used to detect specificity differences between human beings (Goodman, 1959), reactions of only partial identity occurred between the gibbon and the man. In addition, an age-dependent antigenic component occurring as a distinct molecular fraction within the gamma$_2$ globulin of human serum, as was shown by immunoelectrophoresis (Goodman, 1959), was not observed in the monkey or gibbon sera.

These data then support the previously presented theory (Goodman, 1959) concerning the effects of protein antigenicity on human evolution, for it was predicted that the proteins arising late in the developmental phase of ontogeny will show more intra- and inter-specific antigenic variation than those arising early. Gamma globulin, of course, arises late in vertebrate ontogeny whereas albumin arises early. The verification of the theory's prediction was not surprising, since the type of protein variation observed is exactly what we would expect on viewing the molecular substratum of organisms from the perspective of the law of parallel embryological development. Rather the significance of the view which leads to this prediction is that it shows how the persistence of parallel states of embryological development in mammals would be ensured by the operation of immune mechanisms.

ACKNOWLEDGMENTS

The generous cooperation of Dr. George B. Rabb, Curator of the Chicago Zoological Park, in sending samples of animal sera is greatly appreciated.

I am most grateful to Mrs. Mira Wilson for making the notebook of Dr. Morris Wilson available to me and for her permission to reproduce the photographs presented in figure 1. The aid of Andrew Wilson in the performance of the agar precipitin analyses with the chicken antisera is also appreciated. This work was supported in part by research grant M-2476 of the National Institutes of Mental Health.

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TRANSPLANTATION OF *RANA PIPIENS* (*KANDIYOHI* DOMINANT MUTANT) NUCLEI TO *R. PIPIENS* CYTOPLASM

Populations of *Rana pipiens* in Minnesota and Wisconsin contain individuals which have a vermiculate mottled color pattern. Weed (1922) described the frog as a new species and designated it *Rana kandiyobi*. Breeding tests of the "mottled" leopard frog by Volpe (1955) have shown that it differs from the common leopard frog, *Rana pipiens*, by a single dominant gene that influences color pattern and therefore should be referred to as *Rana pipiens* (*kandiyobi* dominant mutant). Although the breeding experiments of Volpe gave no evidence of a maternal cytoplasmic effect, it seemed desirable to utilize the technique of nuclear transplantation to study the effect of foreign (that is, non-*kandiyobi*) cytoplasm on the expression of the *kandiyobi* gene.

Kandiyobi frogs purchased from a dealer in Wisconsin, and *Rana pipiens* from the Lake Champlain area of Vermont, were induced to ovulate by pituitary injection (Rugh, 1934). Animal hemisphere blastula nuclei from normally fertilized eggs (*kandiyobi* female × *kandiyobi* male) provided donor nuclei for transplantation into activated, enucleated *R. pipiens* eggs (Briggs and King, 1952, 1953).

A total of 68 nuclear transplantations were attempted. Of this number, 21 showed no cleavage, 17 resulted in partial or abnormal cleavage, and 30 had a normal cleavage pattern. There were ten normal-appearing gastrulae, half of which developed to larval stages. Three underwent metamorphosis and formed juvenile frogs. Each of the juvenile frogs displayed a pigment

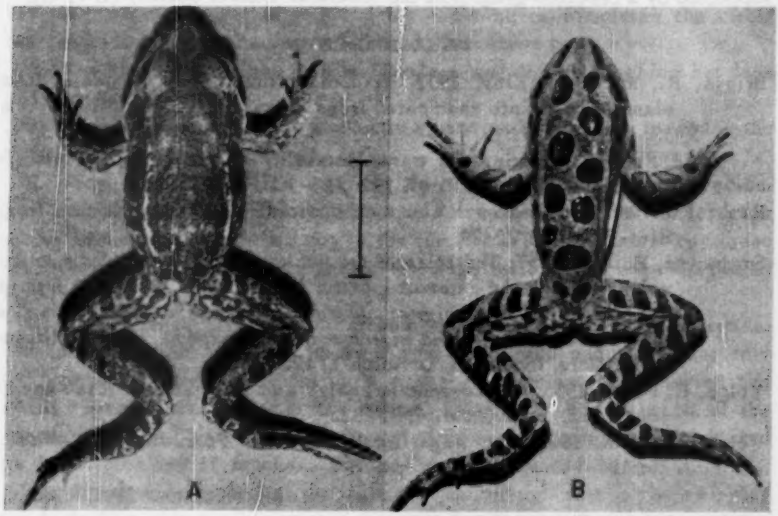

FIGURE 1. A. Juvenile mottled leopard frog produced by transplantation of Wisconsin *kandiyobi* nucleus to Vermont *R. pipiens* cytoplasm. B. Juvenile Vermont *R. pipiens*. Length of index line 10 mm.

pattern similar to that of the *kandiyobi* frog. A juvenile mottled frog produced by nuclear transplantation may be compared with an ordinary juvenile leopard frog in figure 1.

The *kandiyobi* frogs produced by nuclear transplantation appeared in no way different in their development from ordinary *R. pipiens*. The fact that they were indeed *kandiyobi* frogs was not obvious until metamorphosis neared completion. The juvenile adults showed characteristic mottlings or irregular flecks of pigment between spots on the sides and on all four legs. The extent of mottling on the backs varied—one of the frogs had more back mottling than the other two.

Similarly, Sambuichi (1957) transferred blastulae nuclei of one subspecies of *R. nigromaculata* to another. One hybrid tadpole neared metamorphosis from a total of 262 operated eggs. Sambuichi reported that the tadpole displayed morphological characteristics of the nuclear-donor subspecies.

From the present study, it appears that cytoplasm from wild type *R. pipiens* has no obvious effect on the expression in pigment pattern of the *kandiyobi* gene. Since the combination of nuclei and cytoplasm used results in viable progeny, it is suggested that such mutant frogs could be useful as a source of nuclei for transplantation tests requiring an easily recognizable genetic marker associated with the donor nuclei.

ACKNOWLEDGMENTS

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A PRELIMINARY STUDY OF MATING BEHAVIOR IN *DROSOPHILA PAULISTORUM*

"Sexually active animals find their potential mates and recognize them as belonging to the same species with the aid of stimuli that are but poorly known." (Mayr and Dobzhansky, 1945). However, in the Drosophilidae, some of the stimuli have been carefully identified and their sequence plotted by Spieth (1951, 1952), who observed the mating behavior in 111 species, among them 12 copulas within the species, *D. paulistorum*. Since it is now known that *D. paulistorum* actually represents a cluster of incipient species (Dobzhansky and Spassky, 1959), the courtship characteristic of the different subspecies warrants investigation.

For the purposes of a preliminary study three of the six known subspecies were used, namely the Centro-American, the Amazonian, and the Andean-South Brazilian. Completely sterile male hybrids are produced in crosses between these three subspecies. Spieth's direct observation technique was used, and mature, unetherized males and females were observed in glass chambers. Fifty completed courtships were analyzed and timed by stopwatches, as were many other pairs where courtship did not lead to mounting by the males, but to rejection by the females instead. A total of 214 flies were observed (116 males and 98 females). While a more extensive investigation encompassing all of the subspecies as well as the inter-subspecific hybrids is being carried out, certain generalizations about the mating behavior in *Drosophila paulistorum* are now possible.

Courtship is brief and consists of four distinct elements:

1. Circling—the male attracts the attention of the female, and limits her movements, by running around her. He never completes the circle but reverses his direction every 330° and often stops to

2. Tap—using his fore- and middle legs and lightly touching her legs and abdomen.

3. Licking—involves the proboscis of the male and just precedes the rush-in for the mount.

4. Wing vibrations—this may not be very important in this species. The male frequently flutters only one wing and uses the other for leverage as he raises himself on the body of the female. Vibrating is not continued for more than a few seconds except when the male is interrupted by other flies.

On the average, copulation extends for 17 minutes and 12 seconds. The shortest span occurred in the Amazonian group and lasted for only two minutes. The female involved was then immediately dissected and motile sperm were found in the ventral receptacle and in each of the spermathecae. The longest copula, also in the Amazonian group, lasted for 31 minutes and 11 seconds. Obviously, the range is considerable.

The female rids herself of the male by "switching" that is, by swinging her body from side to side so that the male loses his balance. She rejects a male by lowering her head and elevating the tip of her abdomen high

into the air, so that the vaginal orifice is inaccessible to him. This position is then maintained for several minutes.

Males will orient toward and will court any fly, regardless of sex. Homosexual activity is more likely to occur and with greater intensity, if heterosexual activity has not been climaxed by the ejaculation of sperm. The procedure is identical in both cases, and homosexual courtship may progress to the point where one male attempts to mount another. One sees chains of three or more flies, made up of a female at the front being courted by a male who is in turn being courted by other males. The longest chain seen consisted of five flies, one female and four males, but this one quickly broke up.

If the speed and persistence of males belonging to the three subspecies are compared, there is ostensibly a pronounced difference in sexual drive. This is somewhat like the differences in the geographic strains of *Drosophila prosaltans* (Dobzhansky and Streisinger, 1944) where certain races are sexually more excitable than others. In order of decreasing sexual activity in *D. paulistorum* we have: 1) the Amazonian subspecies, 2) the Andean-South Brazilian subspecies, and 3) the Centro-American subspecies. Males of the Amazonian group have inseminated all of the females by one hour after introduction into the observation chambers. Courtship begins immediately after introduction. Andean-South Brazilian males take somewhat longer to accomplish this. (Most homosexual activity is observed in this group.) The Centro-American males, however, require approximately eight hours to mount all of the females present, and they have been repeadly watched for this length of time. None of these males will court two females concurrently, and the courtship is always very slowly paced.

The recording of behavioral differences between subspecies at the "mechanical and physical action levels" only reflects more profound differences at the sensory and physiological levels. This is crucial in view of the reproductive isolation that is being evolved between incipient species.

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